Volume 43 February 1957 Number 2

Proceedings of the National Academy of Sciences

of the United States of America

The Proceedings of the National Academy of Sciences

OFFICERS OF THE ACADEMY

DETLEV W. BRONK
President

GEORGE W. CORNER
Vice President

JOHN G. KIRKWOOD
Foreign Secretary

HUGH L. DRYDEN
Home Secretary

WILLIAM J. ROBBINS
Treasurer

EDITORIAL BOARD OF THE PROCEEDINGS

WENDELL M. STANLEY
Chairman

HUGH L. DRYEEN, *Home Secretary*
WILLIAM J. ROBBINS, *Treasurer*

JOHN G. KIRKWOOD, *Foreign Secretary*
EDWIN B. WILSON, *Managing Editor*

J. W. BEAMS	G. W. BEADLE	GREGORY BREIT
R. E. CLELAND	E. A. DOISY	L. C. DUNN
W. W. RUBEY	G. C. EVANS	W. F. LIBBY
HARLOW SHAPLEY	C. H. GRAHAM	J. VON NEUMANN
F. E. TERMAN	J. T. PATTERSON	PAUL WEISS

Subscription price is $12.50 per volume (12 issues); the price of a single issue is $1.25. Make all remittances payable to The University of Chicago Press in U.S. currency or its equivalent. Authorized agent for the British Commonwealth, except North America and Australasia: Cambridge University Press, Bentley House, 200 Euston Road, London, N. W. 1, England.

Editorial correspondence should be addressed to the PROCEEDINGS of the NATIONAL ACADEMY OF SCIENCES, Attention Miss Mary D. Alexander, University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois.

Business correspondence should be addressed to the Journals Division, University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois.

Subscribers are requested to notify The University of Chicago Press and their local postmaster immediately of change of address.

Microfilms of complete volumes of this journal are available to regular subscribers only and may be obtained at the end of the volume year from University Microfilms, 313 N. First Street, Ann Arbor, Michigan.

Entered as second-class matter June 21, 1920 at the Post-office at Easton, Pennsylvania, under the Act of August 24, 1912. Accepted for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized June 18, 1920.

PRINTED IN THE U. S. A.

THE PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES
is published for
THE NATIONAL ACADEMY OF SCIENCES
by
THE UNIVERSITY OF CHICAGO PRESS

Proceedings of the
NATIONAL ACADEMY OF SCIENCES

Volume 43 · Number 2 · February 15, 1957

*THE METABOLISM OF L-THREONINE AND GLYCINE BY ESCHERICHIA COLI**

By Dorothy A. Miller† and Sofia Simmonds

DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Communicated by Joseph S. Fruton, November 28, 1956

The ability of strains of *Escherichia coli* to synthesize glycine from an exogenous source of threonine has been reported by several investigators.[1–3] This synthesis might result, as it does in higher animals, from the cleavage of threonine to glycine and acetaldehyde (cf. Lin and Greenberg[4]). Data pertaining to this problem were obtained during the study of serine-glycine auxotrophs of *E. coli* strain K12 and form the subject of this communication.

One of the auxotrophs used (strain S/G) requires for growth an exogenous source of either L-serine or glycine.[5] As shown by isotope experiments,[6] cultures of strain S/G grown in the presence of one or the other of the required amino acids derive about 90 per cent of their protein glycine and serine from the amino acid supplied in the medium. Although strain S/G does not grow in the absence of glycine and serine, about 10 per cent of the protein glycine and serine formed during growth in their presence is derived from glucose-carbon, possibly via the intermediate formation of threonine.

Under the conditions generally employed to determine the amino acid requirements of auxotrophs,[5] strain S/G does not use L-threonine in place of L-serine or glycine. However, in a medium containing suboptimal concentrations of L-serine (0.3 millimole per liter) or of glycine (0.2 millimole per liter), the presence of L-threonine permits greater growth than that observed in its absence.[6] Moreover, there is a very slow increase in the absorbancy (optical density) of the culture and in the number of viable cells if the basal medium[5, 7] supplemented only with L-threonine is inoculated with a sufficiently large number of cells (*ca.* 5×10^8 cells/7 ml. of medium). Repeated serial passage of the resultant culture through a threonine-containing medium gives rise to a new, stable strain that behaves like a serine-glycine-threonine auxotroph (Table 1); this strain has been named "S/G/T."

As a working hypothesis, it was assumed that strain S/G/T grows readily on L-threonine because it can derive the required glycine (and serine) from the four-carbon amino acid. The experiments reported below provide support for his hypothesis.

TABLE 1

GROWTH RESPONSE OF STRAINS S/G AND S/G/T

AMINO ACID SUPPLEMENT	mM CONCENTRATION OF AMINO ACID REQUIRED FOR MAXIMAL OPTICAL DENSITY OF 0.15 IN 24 HOURS*	
	Strain S/G/T	Strain S/G
L-Serine	0.06	0.46
Glycine	0.10	0.37
L-Threonine	0.16	No growth

* Each 7-ml. portion of medium was inoculated with *ca.* 3×10^7 cells and incubated on a shaker at 35°. The concentrations of each amino acid (in millimoles per liter) shown in the table were estimated from curves obtained by plotting the optical densities at the end of the growth cycles against the initial concentrations of the amino acid in the medium (see J. O. Meinhart and S. Simmonds, *J. Biol. Chem.*, **213**, 329, 1955).

EXPERIMENTAL

Preparation of Lyophilized Cells.—Strains S/G/T and S/G were grown under forced aeration for 23 hours at 38° in a liquid medium containing 10 gm. of Bacto yeast extract and 6 gm. of Bacto peptone dissolved in 2 liters of distilled water. The cells were harvested by centrifugation, washed twice with 0.9 per cent NaCl, and suspended in 20 ml. of distilled water. The cell suspension was frozen and lyophilized, and the dry solid (*ca.* 1.6 gm.) was powdered in a mortar and stored in a freezer.

Isotope Experiments.—Each incubation mixture contained 30 mg. of lyophilized cells (*ca.* 1.8 mg. of bacterial nitrogen), 6 micromoles of glycine, and 30 micromoles of L-threonine plus sufficient uniformly labeled L-threonine (Nuclear Instrument and Chemical Corporation, Chicago) to provide the desired amount of C^{14}; these constituents were present in a total volume of 3 ml. of 0.02 *M* phosphate buffer, pH 7.5.

The mixtures were incubated in stoppered tubes on a shaker at 35°. After 2.5 hours, 1 ml. of the mixture (solution A) was removed for use in the isolation of keto acids as described below. To the remainder of the incubation mixture was added 0.2 ml. of a trichloroacetic acid solution (100 gm/100 ml), and, after 30 minutes, the mixture was centrifuged and the supernatant fluid decanted (solution B). The precipitate was washed with distilled water and centrifuged again, to provide "washed cells."

One and a half milliliters of solution B was extracted with small portions of ether, and the resultant aqueous solution (solution C) was subjected to column chromatography[8] on Dowex-50 (column size, 0.9×30 cm.). The column was eluted with 1 *N* hydrochloric acid (flow rate, *ca.* 2.4 ml/hour) until 205 fractions (fraction volume, 0.6 ml.) had been collected, and then with 0.2 *N* sodium hydroxide until an additional 32 fractions were obtained. The elution was followed by C^{14} analysis of 0.06-ml. samples taken from every third or fourth fraction. The amino acids in the radioactive fractions were identified by paper chromatography in two solvent systems (system I, pyridine-water [65:35]; system II, *tert.*-butanol-methylethylketone-concentrated ammonium hydroxide-water [10:10:3:5][9]).

The fractions containing each amino acid were pooled and concentrated to dryness *in vacuo* over P_2O_5 and NaOH. (The data in Table 2 represent minimal values, since no corrections were made for the amount of C^{14} lost during the analysis of the column eluates.) Carrier glycine and L-threonine (20 mg.) were added to the appropriate samples from the column, and the amino acids were reisolated and twice recrystallized from water by the addition of ethanol.

Solution A was treated with 0.2 ml. of trichloroacetic acid solution, and its volatile constituents were forced by aeration (with CO_2-free air) into a 0.1 per cent

solution of 2,4-dinitrophenylhydrazine dissolved in 2 *N* hydrochloric acid and thence into a saturated solution of barium hydroxide. The barium carbonate was collected by centrifugation, washed with water, and dried. To the phenylhydrazine solution were added carrier acetaldehyde (8 mg.) and an excess of 2,4-dinitrophenylhydrazine and the resultant 2,4-dinitrophenylhydrazone was isolated and twice recrystallized from hot absolute ethanol by the addition of water. The remainder of solution A was centrifuged, and carrier α-ketobutyric acid (10 mg.) and excess 2,4-dinitrophenylhydrazine were added to the supernatant fluid; the 2,4-dinitrophenylhydrazone was isolated and twice recrystallized.

All samples were plated, at "infinite thinness," on aluminum planchets, and their radioactivity, corrected for background, was determined in a gas-flow counter in the Geiger region.

Deamination Experiments.—The composition of the incubation mixtures and the experimental conditions were similar to those described for the isotope experiments. At the end of the incubation, the mixtures were centrifuged, and the ammonia content of the supernatant fluid was determined by a modified Conway procedure.[10]

Two-dimensional Paper Chromatography.—0.05 ml. portions of solution B from the isotope experiments and of the supernatant fluid from the deamination experiments were examined. Solvent system I has used to develop the chromatograms in the first dimension, and solvent system II for the second dimension. The chromatograms were sprayed with ninhydrin to detect amino acids, and, in the isotope experiments, chromatograms also were examined by radioautography[11] (time of exposure to X-ray film, 2 months).

RESULTS AND DISCUSSION

As may be seen from the data in Table 2, little C^{14} is incorporated into the "washed cells" by either strain. A small amount of the C^{14}-threonine degraded by the bacterial preparations can be accounted for as α-ketobutyric acid, which, presumably, is formed by the action of L-threonine dehydrase.[12] Strain S/G catabolizes threonine less rapidly than does strain S/G/T, but it forms significantly more α-ketobutyric acid.

TABLE 2

RESULTS OF ISOTOPE EXPERIMENTS

Material Analyzed	Strain S/G/T		Strain S/G	
	Total C^{14} in C.P.M.*	Per Cent of Initial C^{14}	Total C^{14} in C.P.M.*	Per Cent of Initial C^{14}
Initial incubation mixture	2.34×10^6	...	2.05×10^6	...
"Washed cells"	1.06×10^5	4.5	1.19×10^5	5.8
Supernatant fluid (solution B)	1.71×10^6	73	1.15×10^6	56
Threonine†	7.43×10^5	32	1.01×10^6	49
Glycine†	2.74×10^5	12	...	0
α-Ketobutyric acid‡	1.88×10^4	0.8	5.73×10^4	2.8
Acetaldehyde‡	0	0	0	0
CO_2‡	3.12×10^4	1.3	*Ca.* 390	*Ca.* 0.02
Two-dimensional paper chromatograms:				
Ninhydrin-positive amino acids	Threonine and glycine		Threonine	
Radioactive amino acids	Threonine and glycine		Threonine	

* Calculated for the entire incubation mixture.

† Combined fractions from column chromatography of solution C. Analysis of samples isolated after the addition of carriers indicated that for strain S/G/T at least 88 per cent, and for strain S/G at least 97 per cent, of the C^{14} in the column eluates was present as the amino acids.

‡ Analyzed as derivatives isolated from solution A (see text).

In the isotope experiment with strain S/G/T, the elution diagram obtained by column chromatography showed two major radioactive peaks; the first, and larger, peak corresponded to threonine, and the second peak to glycine. C^{14} analysis (Table 2) indicated that at least 24 per cent of the carboxyl- and α-carbon atoms of labeled threonine supplied to strain S/G/T is converted to glycine. The mechanism by which this conversion occurs is unclear, since no radioactive acetaldehyde was detected.

Unlike strain S/G/T, strain S/G apparently does not form glycine from labeled threonine. The elution diagram from the experiment with strain S/G showed only one radioactive peak, corresponding to threonine. Although it is possible that strain S/G converts threonine to glycine and then immediately catabolizes the glycine, this possibility is rendered unlikely by the fact that no major radioactive compound (other than threonine) was detected by any of the analytical procedures employed.

In the isotope experiment with strain S/G, paper-chromatographic examination of all the fractions collected subsequent to the elution of threonine from the column showed them to be devoid of glycine. The observation that strain S/G destroys all the glycine initially present in the incubation mixture was unexpected, since resting cell suspensions of strains S/G and S/G/T had been found to deaminate glycine at the same rate.[6] Consequently, the ability of the two strains to produce ammonia from L-threonine, from glycine, and from a mixture of the two was examined under conditions similar to those used in the isotope experiments. Again, lyophilized preparations of the two strains were found to deaminate glycine at the same rate. With strain S/G, the amount of ammonia formed (4.70 micromoles) from the mixture of 2 micromoles of glycine and 10 micromoles of L-threonine is equal to the sum of the ammonia formed from glycine alone (1.49 micromoles) plus that formed from threonine alone (3.11 micromoles). In agreement with the results of the isotope experiment, after strain S/G has acted on the mixture, no glycine can be detected by paper chromatography in the incubation mixture. On the other hand, paper-chromatographic analysis indicates that the action of strain S/G/T on the amino acid mixture does not cause the complete destruction of glycine. It is also of interest that strain S/G/T produces much less ammonia (5.44 micromoles) from the amino acid mixture than would be expected on the basis of the ammonia produced when each amino acid is tested separately (1.50 micromoles from glycine and 4.90 micromoles from threonine).

The data from the isotope and deamination experiments suggest that the presence of L-threonine may inhibit the destruction of glycine by strain S/G/T but not by strain S/G. Whether these conclusions have any bearing on the threonine-glycine relationships in growing cultures is uncertain. However, it is possible that strain S/G/T can grow on threonine not only because of the ease with which it converts threonine to glycine but also because preformed threonine may inhibit the deamination of newly formed glycine.

Until similar isotope and deamination experiments have been carried out with the wild-type strain K12, it is not possible to decide which, if either, of the auxotrophs has inherited the metabolic ability of the parent prototroph. Preliminary experiments indicate that lyophilized preparations of strain K12 can effect a very small net synthesis of glycine from an exogenous source of (unlabeled) threonine.

Thus the available data suggest that if growing cultures of *E. coli* synthesize glycine from endogenous threonine, the synthesis would not be sufficiently rapid to support the growth of auxotrophs unable to derive glycine and serine by another pathway. This is clearly the situation that obtains during the growth of serine-glycine auxotrophs of strain K12.[5, 6]

SUMMARY

A study has been made of the ability of lyophilized preparations of a serine-glycine auxotroph and of a serine-glycine-threonine auxotroph of *E. coli* strain K12 to form glycine from L-threonine. Only the strain capable of growing on L-threonine was found to effect this synthesis under the experimental conditions employed.

* This study was aided by grants from the Nutrition Foundation and the Rockefeller Foundation. The data presented were taken in part from a dissertation presented by Dorothy A. Miller to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy.

† Nutrition Foundation Fellow, Yale University.

[1] J. M. Ravel, R. E. Eakin, and W. Shive, *J. Biol. Chem.*, **172**, 67, 1948.

[2] R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten, *Studies of Biosynthesis in Escherichia Coli* (Carnegie Institution of Washington Publication No. 607 [Washington, D.C., 1955]).

[3] F. Gibson, M. J. Jones, and H. Teltscher, *Biochem. J.*, **64**, 132, 1956.

[4] S. C. C. Lin and D. M. Greenberg, *J. Gen. Physiol.*, **38**, 181, 1954.

[5] J. O. Meinhart and S. Simmonds, *J. Biol. Chem.*, **213**, 329, 1955.

[6] D. A. S. Miller, Ph.D. thesis (cf. ref. 42 in these PROCEEDINGS, **42**, 694, 1956), Yale University, 1956.

[7] S. Simmonds, M. T. Dowling, and D. Stone, *J. Biol. Chem.*, **208**, 701, 1954.

[8] W. H. Stein and S. Moore, *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 179, 1949.

[9] G. R. Wyatt, T. C. Loughheed, and S. S. Wyatt, *J. Gen. Physiol.*, **39**, 853, 1956.

[10] R. B. Johnston, M. J. Mycek, and J. S. Fruton, *J. Biol. Chem.*, **185**, 629, 1950.

[11] The authors are indebted to Mr. Paul J. Rosenbaum, Department of Radiology, Yale University School of Medicine, for his assistance with the radioautography.

[12] Cf. H. E. Umbarger and B. Brown, *J. Bacteriol.*, **71**, 443, 1956.

---

*STERILE INDUCTION OF CROWN-GALL TUMORS ON CARROT TISSUES IN VITRO**

BY RICHARD M. KLEIN AND JOHN L. KNUPP, JR.

NEW YORK BOTANICAL GARDEN, NEW YORK, AND CHEMICAL ENGINEERING DEPARTMENT, UNIVERSITY OF NEW MEXICO, ALBUQUERQUE, NEW MEXICO

Communicated by William J. Robbins, December 13, 1956

INTRODUCTION

Studies on the etiology of crown gall have shown that the transformation of normal plant cells into crown-gall tumor cells may be divided into at least three phases, each requiring the action of chemical agents on the affected cell.[1] The first of these phases, *conditioning*, involves the activation of healthy cells by agents usually present in plant sap. Once conditioned, cells are acted upon by a tumor-inducing principle (T-iP) which appears to be a metabolic product of virulent crown-gall

bacteria. The incipient tumor cells resulting from this *induction* by T-iP are *promoted* during the third phase by growth substances of the indoleacetic acid type which are derived either from the bacteria or from the affected cells. Elimination of any of these steps, alterations of their sequence, or modifications in the presentation time of their action prevents or severely impairs the induction and development of tumor cells.

The nature of the factors required for conditioning, although currently unidentified, are under investigation, and the necessity for indoleacetic acid in tumor-cell promotion has been demonstrated. The composition of T-iP, on the other hand, is still obscure. As an introduction to its characterization, it became necessary to obtain its synthesis by the bacteria in vitro and to develop a quantitative bio-assay for its activity. Klein[2] reported that in vitro bacterial synthesis of T-iP occurs in a medium containing unheated plant sap but not in heated sap or in synthetic medium. This preliminary report was qualitatively confirmed by Manigault *et al.*,[3] who found in addition that T-iP could be concentrated by high-speed centrifugation and was inactivated by deoxyribonuclease (DNase).

Since the appearance of our initial report, more precise in vitro systems for studying the synthesis and activity of T-iP have been developed. Although the work is still in progress, the general techniques—as well as some specific findings relative to tumor-cell formation—are reported in this paper.

GENERAL TECHNIQUES

Pretreatment.—Disks of young secondary phloem from carrot roots known to react strongly to tumor formation (designated "SR disks"[4]) were isolated aseptically.[5] The disks were allowed to heal by placing them, cambial-adjacent side up, on the surface of White's tissue-culture medium supplemented with 10^{-7} gm/l naphthaleneacetic acid for three days, a period determined to give optimal results in subsequent treatment. Disks healed on unsupplemented White's agar or on medium supplemented with coconut milk showed less tumor formation following sterile tumor induction. The disks were then exposed to agents known to be active in tumor-cell formation by transferring them into sterile test tubes containing these agents. In all reported experiments, the proper etiological sequence of exposure, i.e., conditioning, induction, and promotion, was maintained. Unreported experiments in which steps were eliminated or the order of exposure was altered invariably resulted in the failure of treated disks to form tumors. The incubation temperature was maintained at 26°–27° C. Sterility checks were routinely made into nutrient broth.

Conditioning.—Healed disks were transferred into sterile test tubes containing 1.5–2.0 ml. of plant sap and conditioned[6] for 24 hours. The conditioning factor(s) was not species-specific. The sap of numerous plants, including beet, parsnip and carrot roots, potato tubers, and herbaceous stems, contains this factor. The conditioning factor was not found in extracts of malted barley.

When carrot roots were used as the source of the required factor, the juice was expressed from roots known, by previous bio-assay, to react strongly to tumor formation, since the conditioning factor is absent or in suboptimal amounts in carrots that react weakly to tumor formation.[4] The sap was expressed in a Carver hydraulic press after macerating the tissues in a meat chopper. It was clarified at 10,000 × *g*, filtered through a No. 01 Selas porcelain filter candle, dialyzed against

0.001 *M* phosphate buffer, pH 7.0, to remove some unidentified toxic substance that causes browning of the tissues, and finally sterilized by successive passages through Nos. 015, 02, and 03 Selas filter candles.

Induction.—The unheated plant sap or extract required for the bacterial synthesis of tumor-inducing principle (containing a factor(s) designated "T-iP synthesis factor") was dialyzed against 0.001 *M* phosphate buffer, pH 7.0, glucose was added to a final concentration of 1.0 per cent, and the sap was sterilized by filtration through Selas filter candles. Fifty- to 75-ml. aliquot portions were inoculated with *ca.* 10^8 cells of a log phase nutrient-broth culture of *Agrobacterium tumefaciens* strain B6.[5] The culture was shaken at 26° C. for 12–18 hours, clarified by centrifugation, dialyzed for several hours against 0.001 *M* phosphate buffer, pH 7.0, to remove toxic components, and finally sterilized by filtration. The T-iP synthesis factor was not host-specific and was present in beet sap and other plants. It was present in both weak and strong reactor carrots. A freshly prepared extract of malted barley was the best source tested for the T-iP synthesis factor (Table 1). It was prepared by grinding malted barley in three times its volume of 0.001 *M* phosphate buffer, pH 7.0, in a Waring Blendor and collecting the supernatant after centrifugation. The use of carrot sap or extract of malted barley diluted below 1:1 resulted in a less active preparation of T-iP.

The healed and conditioned carrot disks were transferred to test tubes containing 2.0 ml. of sterile culture fluid containing the T-iP and were incubated for 24 hours. The induction step was repeated twice at 24-hour intervals using freshly prepared T-iP (Table 2). More frequent renewals might be better, since crown-gall bacteria undoubtedly synthesize T-iP in the host plant constantly as long as the synthesis factor is available. The T-iP in the culture fluid was inactivated at 8° C. in its present crude state within 18–24 hours.

TABLE 1

SOURCE OF THE T-iP SYNTHESIS FACTOR AFFECTING THE BACTERIAL SYNTHESIS OF TUMOR-INDUCING PRINCIPLE

	Percentage of Disks Forming Crown-Gall Tumors*		Percentage of Disks Forming Crown-Gall Tumors*
Sap from tumor-susceptible carrot roots	64	Autoclaved extract from malted barley	0
Sap from tumor-insusceptible carrot roots	64	Commercial malt extract	0
Sap from beet roots	55	Sap from carrot roots heated to 80° C. for 10 min.	29
Extract from malted barley	75		

* All disks previously conditioned and subsequently promoted.

TABLE 2

TIME OF CONTACT OF TUMOR-INDUCING PRINCIPLE WITH CONDITIONED CARROT DISKS AFFECTING THE FORMATION OF TUMORS*

Hours	Percentage of Disks Forming Crown-Gall Tumors†	Hours	Percentage of Disks Forming Crown-Gall Tumors†
0	0	72	70
24	13	96	46
48	40		

* Tumor-inducing principle freshly prepared for each 24-hour period.
† All disks previously conditioned and subsequently promoted.

Promotion.—Indoleacetic acid, or compounds with similar biological activity, seem to be required to promote T-iP-treated cells into primary tumor cells.[7, 8] It has been determined that 1–5 $\times$ 10^{-6} M indoleacetic acid at pH 7.0 acting for 48–72 hours gave optimal results. The healed, conditioned, and T-iP-treated carrot disks were transferred into test tubes containing 1.5 ml. of indoleacetic acid for this period of time.

Growth and Isolation of Tumor Tissue.—Following the treatments leading to tumor-cell formation, the disks were transferred, cambial-adjacent side up, to tubes of unsupplemented White's tissue-culture medium. After 20–30 days of incubation, the percentage of disks showing crown-gall development was determined. Since callus formation on carrot phloem is very slight on this medium,[5] visual inspection was satisfactory.

We believe that the tumors which developed on carrot disks following the above procedure are true crown-gall tumors. When excised from the carrot disks and transferred to fresh, unsupplemented White's medium, the tumors continued to proliferate and have been carried for over 19 monthly transfers without significant reduction in their rates of growth. Callus tissue, unless grown on a medium containing growth substances (indoleacetic acid, coconut milk), ceases to grow after two to three transfers. In no experiment, however, did the rates of growth of the sterile crown-gall tumors approach that of carrot tumors induced by the bacteria. The excised crown-gall tumors were insensitive to or inhibited by a wide range of indoleacetic acid and naphthaleneacetic acid supplements and by coconut milk but were never stimulated. This is characteristic of many crown-gall tumors and is distinctly atypical of callus tissues of carrot. Another available criterion for crown-gall tumor tissues, i.e., the ability of the tissue to grow autonomously as tumor tissue when grafted into healthy tissue,[9] has not been fulfilled for these sterile carrot tumors, although we had demonstrated graft autonomy with tomato.[2] Preliminary grafting experiments have been inconclusive because of technical difficulties arising in attempts to graft tumor tissues into the tissues of carrot roots. Experiments utilizing other species of *Daucus* possessing graftable stems are contemplated.

DISCUSSION

The general technique outlined above has regularly resulted in 60–80 per cent of the treated disks forming crown-gall tumors. We have not, however, obtained 100 per cent induction in any experiment. During the development of this procedure we have increased the percentage of disks forming tumors from 25–35 per cent to its present value. Since all disks were capable of tumor formation if inoculated with crown-gall bacteria, further modification of the technique is necessary.

Several additional significant findings on tumor induction have been made. Of primary importance is the inactivation of T-iP by DNase (Table 3), a finding that confirms Manigault's note[3] and reinforces our earlier, indirect evidence that it is a deoxyribonucleic acid.[11, 12] Since it may be collected by centrifugation,[3] is nondialyzable, and its general chemical composition is now known, the eventual isolation and purification of T-iP may be in sight.

Our experiments show that a conditioning step using plant juice is an absolute requirement for tumor formation on unwounded or healed tissues and suggests that

the juice contains some factor(s) acting specifically during this phase. The increase in the percentage of tumors formed when the disks were healed on a medium containing naphthaleneacetic acid relative to the tumor formation on disks healed without growth-substance supplements suggests that conditioning may require several etiological agents. The sources and properties of the factors in plant sap required for condititioning and of those for the synthesis of T-iP show that they are not identical. Their chemical nature is currently under investigation.

TABLE 3

STABILITY OF T-iP SYNTHESIZED IN PRESENCE OF CARROT SAP

Treatment	Percentage of Disks Forming Crown-Gall Tumors*	Treatment	Percentage of Disks Forming Crown-Gall Tumors*
Control	67	Heat treatment of T-iP:	
Deoxyribonuclease treatment, 0.1 mg/ml for 60 min.	0	34° C. for 30 min.	67
		50° C. for 60 min.	0
Supernatant remaining after acid (pH 5.0) precipitation of protein	58	100° C. for 5 min.	0
Synthesis of T-iP at 34° C.	0		

* All disks previously conditioned and subsequently promoted.

It has been postulated that the failure of tumors to form on inoculated plants held at 32°–33° C. rather than at 25° C. was due to the heat lability of the T-iP molecule.[10] Our results indicated that T-iP is not highly labile at these temperatures but that it is probably not synthesized by the bacteria at the higher temperatures (Table 3).

SUMMARY

A technique has been developed for the in vitro synthesis and bio-assay of the tumor-inducing principle, the pre-eminent factor active in the transformation of normal plant cells into crown-gall tumor cells. This synthesis can occur only in the presence of a heat-labile "synthesis factor" present in a number of plants. Both indirect and direct evidence suggests that the tumor-inducing principle is a deoxyribonucleic acid.

* Work supported in part by a grant from the American Cancer Society, recommended by the Committee on Growth, National Research Council. We are indebted to Mrs. Ida Tenenbaum and Mrs. Grace Groves for technical assistance and to Drs. Deana T. Klein and W. J. Robbins for their advice and criticism.

[1] R. M. Klein and G. K. K. Link, *Quart. Rev. Biol.*, **30**, 207–277, 1955.
[2] R. M. Klein, *Brookhaven Symposia Biol.*, **6**, 97–114, 1954.
[3] P. Manigault, A. Comandon, and P. Slizewicz, *Ann. Inst. Pasteur*, **91**, 114–117, 1956.
[4] R. M. Klein, these PROCEEDINGS, **41**, 271–274, 1955.
[5] R. M. Klein and I. L. Tenenbaum, *Am. J. Botany*, **42**, 709–712, 1955.
[6] A. C. Braun, *Growth*, **16**, 65–74, 1952.
[7] A. C. Braun and T. Laskaris, these PROCEEDINGS, **28**, 468–477, 1942.
[8] R. M. Klein and G. K. K. Link, these PROCEEDINGS, **38**, 1066–1072, 1952.
[9] A. C. Braun, *Ann. Rev. Plant Physiol.*, **5**, 133–162, 1954.
[10] A. C. Braun, *Am. J. Botany*, **34**, 234–240, 1947.
[11] R. M. Klein, *Am. J. Botany*, **40**, 597–599, 1953.
[12] R. M. Klein, E. M. Rasch, and H. H. Swift, *Cancer Research*, **13**, 499–502, 1953.

*THE STRUCTURE OF ALPHA KERATIN**

By Maurice L. Huggins

RESEARCH LABORATORIES, EASTMAN KODAK COMPANY, ROCHESTER, NEW YORK

Communicated by W. A. Noyes, Jr., November 23, 1956

Keratin in the unstretched (α) form gives X-ray photographs[1, 2] which resemble, in their main features, those from the α forms of the synthetic polypeptides. With most samples, only a few reflections appear, and they are relatively diffuse. This can be attributed to less regularity of structure, resulting from variation in the *R* groups.

One is tempted to assume[3] the α-keratin structure to consist of helices of the 13-atom-ring type,[4, 5] in a close-packed arrangement. There are several difficulties with this assumption, however, of which three will be mentioned.

Difficulty 1.—The assumed structure does not account for the strong meridional reflection, corresponding to an interplanar distance of about 5.2 A. This is somewhat less than the 5.4 A translation in the direction of the helical axis in the Pauling-Corey model. Although strong near-meridian reflections, corresponding to distances slightly less than 5.4 A, would be expected, the intensity on the meridian should be zero.

Difficulty 2.—The calculated density is too low. Taking the 1.49 A meridional reflection[2, 6] as the shift in the fiber direction per residue and the strong 9.8 A reflection (really a composite reflection[2] with principal components at 9.2 and 10.5 A) as having indices 10.0 (relative to the *pseudo* unit for a strictly close-packed structure), the experimental estimates[7, 8] of average residue weights (112–120 for wool, hair, and horn keratin) give a calculated density of 1.13–1.21 gm/cc. The experimental density is 1.32 gm/cc.

Difficulty 3.—Without additional assumptions, the assumed structure does not account for other observed equatorial reflections, including those at 27, 41, and 81 A, or for various strong meridional reflections, corresponding to a unit translation of 198 A.[2, 9, 10]

To account for the 5.2 A meridional reflection, Crick[11, 12] has proposed that α-helices of the 13-atom-ring type are so distorted that two or three of them twist spirally around a common axis. (See also Lang.[13]) Crick's argument that this could make the side chains of adjacent helices fit together better is reasonable. The sequence of the residues and so of the side-chain distribution can reasonably be related to the periodicity of the superhelix. The density difficulty is not eliminated. With regard to Difficulty 3, it seems possible, in principle, to account for the long meridional spacings of the X-ray pattern, but just how this can be done has not been worked out, nor has any explanation been offered for the long spacings of the equatorial pattern. Moreover, a new problem is introduced:

Difficulty 4.—The α-to-β transformation requires, in addition to the breaking and remaking of hydrogen bonds, the uncoiling of each superhelix, against large frictional forces, of the surrounding helices. Also, practically all cystine or other interchain crosslinks between chains in different superhelices would have to be broken in the process. Since cystine crosslinks would not be expected to be broken under these conditions, it must be assumed that all such crosslinks are between chains in the

same superhelix. (About one residue out of nine, on the average, is a "half-cystine," connected to another half-cystine residue. These connections have usually been considered to be between residues in different chains.)

Pauling and Corey[14] have proposed an α-keratin structure in which six distorted 13-atom-ring helices are coiled around one such helix to give a seven-strand cable, these cables and single helices (two for each cable) being placed in a hexagonal *pseudo*-unit cell of edge length 32.4 A. Expected X-ray reflections would include those corresponding to distances of 28.1, 10.6, and 9.4 A, in reasonable agreement with observed reflections at about 27, 10.5, and 9.2 A (MacArthur[2]). The 5.2 A meridian reflection is explained in the same way as by Crick for his model. Side-chain distributions might conceivably account for the longer meridional and equatorial spacings. The calculated density is 1.24–1.32 gm/cc for assumed average residue weights of 112–120 units. The agreement with the observed density (1.32) is only fair, since the average residue weight, in the samples giving the X-ray data, was probably 116 or less. As Pauling and Corey suggest, a small amount of water incorporated in the structure would account for this discrepancy. Also, since Elliott[15] has shown, by infrared spectrum evidence, that natural keratin contains large percentages of amorphous material, together with some having the β structure, mixed with the α material, the discrepancy cannot be considered very serious. Both the density and the average residue weight in the α regions may be different from the over-all averages.

Difficulty 4 applies to the Pauling-Corey model even more than to that of Crick. Uncoiling of the superhelices during the α-to-β transformation would be expected to be very difficult. Unless cystine crosslinks are broken in the process, all such crosslinks must be between chains in the same cable. The single helices could not be crosslinked to any others.

Difficulty 5.—The polypeptide chains, in the Pauling-Corey model, are of three quite different types, as regards the way in which they are surrounded by others. No explanation of this violation of the principle that like groups tend to be surrounded in like manner has been offered.

A new model, which avoids the difficulties which have been cited, is here suggested. Let us, as a first approximation, assume close-packing of (right-handed) polypeptide helices, with the distance between helix axes about 9 A. Instead of assuming that two or three or more of these helices *coil* spirally around a common axis, let us just consider them to be grouped into triplets, with the residue sequence patterns in each polypeptide helix shifted and rotated, relative to its two neighbors in the same triplet, in such a way as to place equivalent residues around a threefold screw axis—the triplet axis. The interchain packing within each triplet would be expected to be more efficient than between chains of different triplets; hence the distances between chain axes within each triplet should be somewhat less than the distances between adjacent chains of different triplets.

Most efficient packing of the triplets can be achieved if they, in turn, are grouped around a common threefold axis to form "triple triplets" or "nonets." The principle that like groups tend to be surrounded in like manner gives two possibilities with regard to the three chains closest to the nonet axis: (1) like residues may be at the same level or (2) like residues may be arranged in regular spirals around the common threefold (screw) axis. The latter alternative would be expected to give

the better packing. Carrying this idea still further leads to the structure represented in Figure 1.

The unit of structure contains 81 chains in three equivalent groups of 27 chains. Taking the unit distance, a, as 95 A (to give the experimental density of 1.32 gm/cc, if the average residue weight is 114 and the average translation per residue is 1.49 A), the calculated distances for forms 10.0, 20.0, 30.0, 63.0, and 90.0 are 82, 41, 27.4, 10.4, and 9.1 A, respectively, in good agreement with the observed reflections (MacArthur[2]) at 81, 41, 27, 10.5, and 9.2 A. Equatorial reflections with other indices would be expected to be weak or to be included in the broad composite reflections observed[1, 2, 14] in the 9–10 and 3–5 A regions.

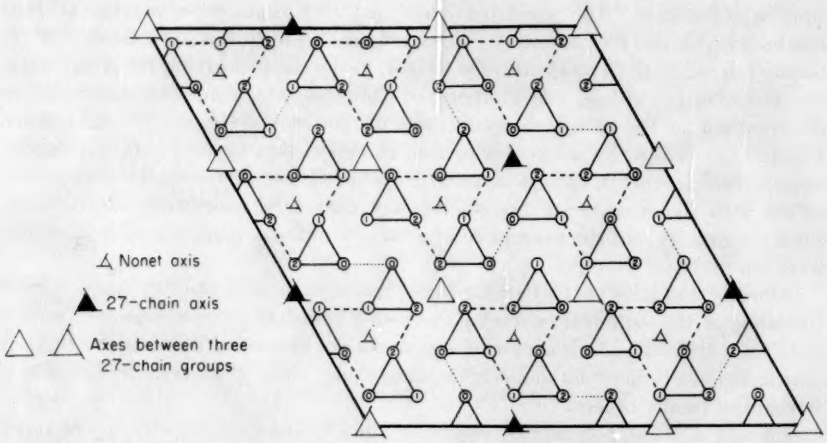


FIG. 1.—Diagrammatic representation of the structure proposed for α-keratin.

In addition to irregularities within each chain, related to differences between the side chains of the various residues, one would expect small shifts of the individual chain axes from their "ideal" positions toward the triplet axes (as already noted) and also a slight twisting of each triplet around the triplet axis (but not a continuous coiling, for reasons which have been mentioned). Similar, but smaller, shifts might occur within the 9-chain and 27-chain groups and around the threefold axes (simple and screw), where three 27-chain groups come together. To these shifts and twists (and probably also to the distribution of crosslinks, to be discussed below) can be attributed the reflections corresponding to the large unit cell. Unless arbitrary assumptions are made as to the magnitudes of the shifts, it is impossible to compute intensities for the different reflections.

The pattern proposed is not incompatible with the coiled-coil proposal of Crick. Each triplet might be a coiled-coil. Perhaps, also, three of these triplets might coil around their common axis and three of the nonet coiled-coiled-coils might do likewise, the rates of twist being suitably co-ordinated with one another. The present proposal, however, does not eliminate the objections to the coiled-coil hypothesis (Difficulty 4). Without continuous coiling, there might still be co-ordinated twisting of the groups of chains for relatively short distances (in the fiber direction), then

some structure irregularity (such as a change in the type of chain helix or an irregularity connected with the insertion of a proline residue) to relieve the interchain stresses resulting from poorer packing or to make interchain crosslinks possible, then further co-ordinated twisting, another structure irregularity, etc., as represented diagrammatically in Figure 2.

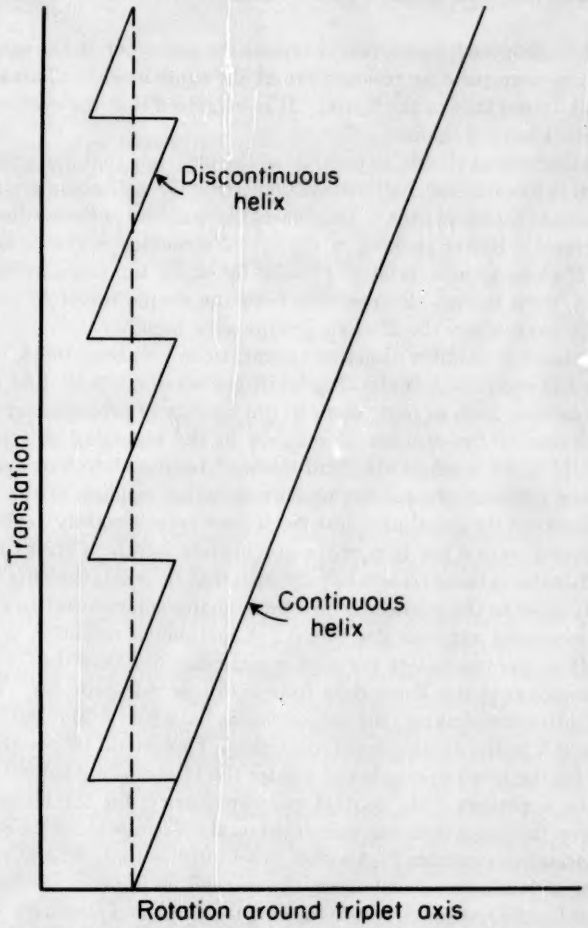


FIG. 2.—Diagram illustrating the discontinuous helix concept.

The relative displacements of the polypeptide chains in the fiber directions, indicated in Figure 1, are such as to make like groups surrounded in like manner, in so far as possible. Other relative displacements might give better over-all packing or better crosslinking between chains, however. The relative heights of like residues may follow a more complicated pattern than that indicated, but the X-ray evidence indicates that the grouping of the chains into triplets, nonets, etc., is maintained.

The pattern shown is based on the assumption that the residue sequences in all chains have the same polarity. This gives better agreement with the principle that like groups tend to be surrounded in like manner than would any pattern which the writer has been able to find, which accounts for the X-ray data. Perhaps this is related to the fact, already mentioned, that in β-keratin the X-ray data seem to indicate a structure with parallel (rather than antiparallel) orientations of the chains.

In the pattern proposed, each chain is adjacent to one other, in the same 27-chain group, in which corresponding residues are at the same height. This is indicated by dashed and dotted lines in the figure. It is suggested that the cystine crosslinks are between such pairs of chains.

In order to conform as closely as possible to the principle that like groups tend to be surrounded in like manner, half the axes centering three 27-chain groups must be simple (non-screw) threefold axes. This places like residues in three adjacent chains on the same level. Better packing in the actual structure might be achieved by shifting the 27-chain groups, relative to each other, by unknown amounts in the fiber direction, even though this destroys both the simple threefold axes and the threefold screw axes, where the 27-chain groups come together.

The unit distance in the fiber direction appears to be [9, 10] about 198 A, corresponding to 133 or 132 residues, if the average shift per residue is 1.49–1.50 A. (Irregularities of structure, such as must occur in the vicinity of proline residues, make a precise calculation of the number of residues in the repeating unit impossible.) About fifteen of these residues are "half-cystine" residues.[7, 8] For cystine crosslinking between adjacent chains, having corresponding residues at the same level, the spacings between the crosslinks must be, at least approximately, multiples of the shift in the fiber direction per turn of the polypeptide spiral. (The expected bond distances and angles in these crosslinks[16] are such that their attachments to the main chains must be close to the points at which those chains come closest to each other.) This line of reasoning suggests that the 5.2 A meridional reflection is due to the crosslinks. If so, the translation per turn is somewhat less than the 5.4 A assumed in the interpretation of the X-ray data from synthetic polypeptides. Perhaps, to give most effective crosslinking, the helical chains have just 3.50 residues per turn, instead of the 3.6 in the synthetic polypeptides. This would be possible, without much strain, for the 13-atom-ring helix[4, 5] or for the 11-atom-ring helix.[17] (It seems likely that the departure from peptide group planarity, for the latter structure, would be less on this basis than was calculated on the assumption of 3.6 residues and 5.29 A displacement per turn.) Another possibility is that, where irregularities (such as those at proline residues) occur, the translation per turn is enough shorter than the value for the regular structure to bring the average down to 5.2 A.

Further details of the structure and its X-ray diffractions depend on the distribution of residues of the different types. Although the approximate relative numbers[7, 8] for several classes of keratin are known, their sequence has not been determined. It is possible, moreover, that the distribution in the crystalline portions, having the α structure, is not the same as the over-all distribution. We do not know whether the proline residues, for example, which constitute about 8 per cent of the total, occur in the crystalline regions (producing structural irregularities, as suggested above) or exclusively or predominantly in the amorphous regions.

* Communication No. 1864 from the Kodak Research Laboratories.

[1] W. T. Astbury and A. Street, *Phil. Trans. Roy. Soc. London*, A, **230**, 75, 1931.
[2] I. MacArthur, *Nature*, **152**, 38, 1943.
[3] L. Pauling and R. B. Corey, these PROCEEDINGS, **37**, 261, 1951.
[4] L. Pauling, R. B. Corey, and H. R. Branson, these PROCEEDINGS, **37**, 205, 1951.
[5] L. Pauling and R. B. Corey, these PROCEEDINGS, **37**, 235, 1951.
[6] M. F. Perutz, *Nature*, **168**, 653, 1951.
[7] G. R. Tristram, in *The Proteins*, ed. H. Neurath and K. Bailey (New York: Academic Press, 1953).
[8] W. H. Ward and H. P. Lundgren, *Advances in Protein Chemistry*, **9**, 243, 1954.
[9] R. S. Bear, *J. Am. Chem. Soc.*, **65**, 1784, 1943.
[10] R. S. Bear and H. J. Rugo, *Ann. N.Y. Acad. Sci.*, **53**, 627, 1951.
[11] F. H. C. Crick, *Nature*, **171**, 59, 1952.
[12] F. H. C. Crick, *Acta cryst.*, **6**, 689, 1953.
[13] A. R. Lang, *Acta cryst.*, **9**, 436, 446, 1956.
[14] L. Pauling and R. B. Corey, *Nature*, **171**, 59, 1953.
[15] A. Elliott, *Textile Research J.*, **22**, 783, 1952.
[16] H. L. Yakel, Jr. and E. W. Hughes, *Acta cryst.*, **7**, 291, 1954.
[17] M. L. Huggins, *J. Am. Chem. Soc.*, **74**, 3963, 1952.

---

*THE STRUCTURE OF COLLAGEN**

BY MAURICE L. HUGGINS

RESEARCH LABORATORIES, EASTMAN KODAK COMPANY, ROCHESTER, NEW YORK

Communicated by W. A. Noyes, Jr., November 23, 1956

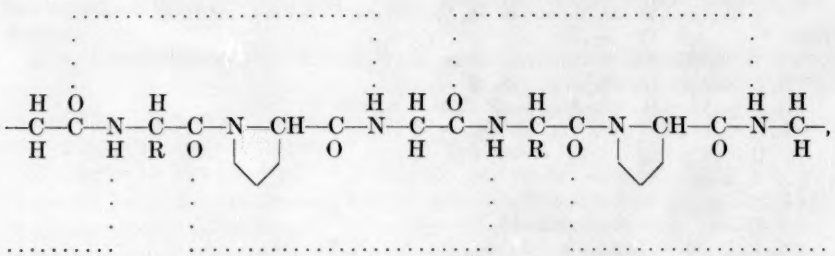
Many models have been proposed for the structure of collagen. The older ones, however, have all been shown to be in definite contradiction with experimental facts.

Recently, Rich and Crick[1] and Ramachandran[2] have made new proposals, involving 3-chain coiled-coils, which avoid some of the difficulties of their earlier models. In the writer's opinion, there is little chance that these new proposals are correct or even approximately so. Since only half the NH groups in each main chain are hydrogen-bonded (unless to unspecified side chains), these structures conform neither to the principle that hydrogen bonds are formed wherever possible nor to the principle that like groups tend to be surrounded by close neighbors in like manner.[3] Also, since pulling out the chains into an extended zigzag arrangement would lengthen the structure by only 25 per cent, there seems to be no way of explaining the large extensions (at least 7- or 8-fold) of the band pattern occasionally observed in the electron microscope pictures.[4]

The writer[5] has proposed a structure which agrees with the primary requirements of the X-ray data,[6] gives maximum hydrogen bonding, permits about $3^1/_2$-fold extension without breaking of primary bonds, and has various other advantages. It has one serious disadvantage, however: half the CO·NH groups have the *cis* orientation. This seems to be in contradiction to the infrared evidence.[7]

A new structure, recently discovered, has only *trans* orientations of the CO·NH groups and seems satisfactory from other points of view, judging from a model made approximately to scale. (Precise co-ordinates have not yet been deduced.)

In conformity with the X-ray data, the polypeptide chain is assumed to be helically coiled, 30 residues per three turns and per 28.6 A, with the residues in groups of three. In the "idealized" structure, each group of three residues contains one glycine residue, one proline or hydroxyproline residue, and one of another type. The pattern of bonds and hydrogen bonds is as indicated in the formula diagram



which shows two three-residue groups, and in Figure 1 *levo* orientations at the asymmetric carbon atoms produce a left-handed spiral.

All the peptide N—H groups are hydrogen-bonded to carbonyl oxygen atoms of other peptide groups. The rings so formed contain 10 atoms each. The N—H and C=O bond orientations appear to be in agreement with the infrared data,[7-10] as far as can be determined from the model.

The analytical data[11] (Table 1) show that very close to one-third of the total number of residues are glycine residues, —NH—CH_2—CO—, in agreement with the

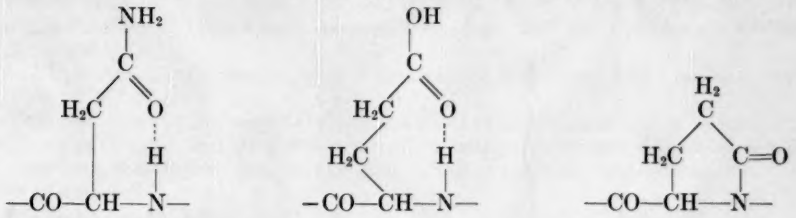
TABLE 1

COMPOSITION OF COLLAGEN

Amino Acid (by Hydrolysis)	—R	Number of Residues per 100 Residues		
Glycine	—H	33.19		
Alanine	—CH_3	9.77		
Valine	—$CH(CH_3)_2$	2.66		
Leucine	—$CH_2CH(CH_3)_2$	3.91		
Isoleucine	—$CH(CH_3)CH_2CH_3$			
Phenylalanine	—$CH_2(C_6H_5)$	1.39		
Methionine	—$CH_2CH_2SCH_3$	0.49		
Serine	—CH_2OH	2.93		
Threonine	—$CH(CH_3)OH$	1.76	5.19	
Tyrosine	—$CH_2C_6H_4OH$	0.50		
Proline	.....	12.00	21.79	
Hydroxyproline	.....	9.78		33.15
Glutamic acid	—$CH_2CH_2CO_2H$	7.04		
Aspartic acid	—CH_2CO_2H	4.32	11.36	
Arginine	—$CH_2CH_2CH_2NHC(NH_2)=NH$	4.52		
Lysine	—$CH_2CH_2CH_2NH_2$	2.81		
Hydroxylysine	—$CH_2CH_2CH(OH)CH_2NH_2$	0.62	8.38	
Histidine	—$CH_2C_3H_3N_2$	0.44		
	Total	100.00		

model. The proline and hydroxyproline residues together constitute only about 22 per cent, but perhaps the residues which yield aspartic acid and glutamic acid

on hydrolysis,[12] when in the native collagen, contain rings which act structurally much like proline and hydroxyproline rings. Such rings might be produced by hydrogen bonding or by condensation reactions:



It is perhaps significant that the sum of the numbers of residues of these four kinds is very near to one-third of the total (Table 1).

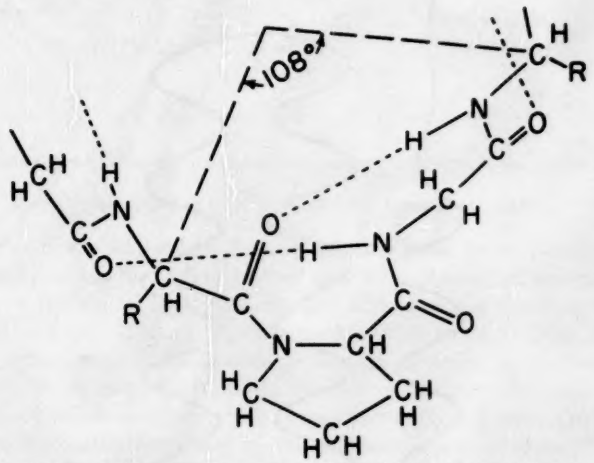


FIG. 1.—Diagrammatic representation of the pattern of bonds and hydrogen bonds in the proposed structure for collagen.

Without producing much disruption of the structure, the —NH—CHR—CO— residue just to the left of a proline residue (see Fig. 1) can also be made a proline or a hydroxyproline residue. This would account for the proline-hydroxyproline sequences which have been found in partial hydrolyzates.[13]

With the model described, the peptide groups can be strictly planar and the N—H···O hydrogen bonds straight. Some other models which have been tried seem reasonably satisfactory otherwise but fail in this regard.

It has already been noted that the band pattern in the electron microscope is occasionally stretched, up to seven or eight times its normal length, i.e., to at least twice the length obtainable by pulling out the proposed helix into an extended zigzag. This extra stretching can be attributed to regions in which the polypeptide chains are irregularly arranged or are arranged in a more extendable pattern, or to a reversal of the directions of some chains or portions of chains, as they are pulled

out. This could result, for example, if the molecules included two or more helices, as indicated diagrammatically in Figure 2. The interhelix connections might be cystine or other crosslinks or bends in the main polypeptide chain, such as might occur where two proline residues (or a proline and a hydroxyproline residue) come together.

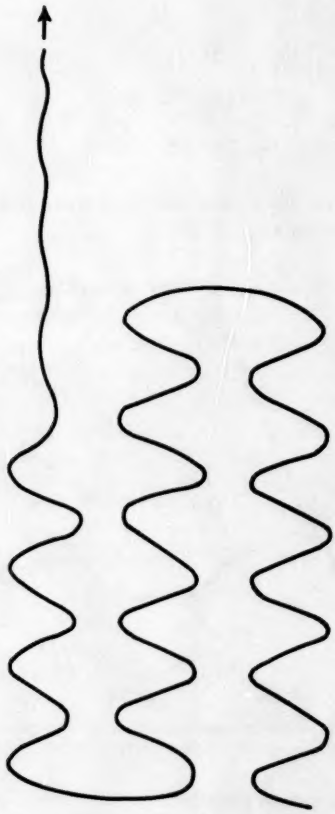


FIG. 2.—Diagram illustrating a possible explanation for the very large band pattern extensions observed in the electron microscope.

The model proposed here of course requires further checking and elaboration. It is presented tentatively as the model which, in the opinion of the writer, is most likely to approximate the true structure.

* Communication No. 1866 from the Kodak Research Laboratories.

[1] A. Rich and F. H. C. Crick, *Nature*, **176**, 915, 1955.
[2] G. N. Ramachandran, *Nature*, **177**, 710, 1956.
[3] M. L. Huggins, *Chem. Revs.*, **32**, 195, 1943.
[4] F. O. Schmitt, C. E. Hall, and M. A. Jakus, *J. Cellular Comp. Physiol.*, **20**, 11, 1942.
[5] M. L. Huggins, *J. Am. Chem. Soc.*, **76**, 4045, 1954.

[6] R. S. Bear, *Advances in Protein Chemistry*, **7**, 69, 1952.

[7] R. M. Badger and A. D. E. Pullin, *J. Chem. Phys.*, **22**, 1142, 1954.

[8] E. J. Ambrose and A. Elliott, *Proc. Roy. Soc. London, A*, **206**, 206, 1951.

[9] G. B. B. M. Sutherland, K. N. Tanner, and D. L. Wood, *J. Chem. Phys.*, **22**, 1621, 1954.

[10] W. E. Seeds, in *The Nature and Structure of Collagen*, ed. J. T. Randall ("Butterworth's Scientific Publications") (London: Butterworth & Co., Ltd.; New York: Academic Press, Inc., 1953), p. 250.

[11] G. R. Tristram, in *The Proteins*, ed. H. Neurath and K. Bailey (New York: Academic Press, Inc., 1953), Vol. 1, Part A.

[12] Analyses show that the amount of amide nitrogen is practically equivalent to the amount of aspartic acid obtained, suggesting that in the native material the aspartic residues are present in amide form, or as an amide condensation product, with the glutamic residues in an acidic form or as an acid condensation product.

[13] T. D. Kroner, W. Tabroff, and J. J. McGarr, *J. Am. Chem. Soc.*, **77**, 3356, 1955.

---

*THE CONTRIBUTION OF THE α-HELICAL CONFIGURATION TO THE OPTICAL ROTATION OF POLYPEPTIDES AND PROTEINS**

BY PAUL DOTY AND R. D. LUNDBERG†

DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS

Communicated by G. B. Kistiakowsky, December 24, 1956

Shortly after it was established that polypeptides could exist in solution as rigid, rodlike α-helices as well as randomly coiled chains,[1] it became evident that the optical rotation of the helical form was substantially more positive than that of the randomly coiled form. For a while it seemed possible to explain this effect either as a result of the change in the environment of the optically active residues in the two configurations or as arising from the existence of a single screw sense in the helical configuration which could then make a contribution to the optical rotation independent of the contribution made by the optically active residues. Within the last year three different experiments have demonstrated that the stable helical configuration is exclusively of one screw sense, that is, right-handed or left-handed. In one experiment it was shown that the rotatory dispersion was normal for the randomly coiled configuration and abnormal for the helical form.[2] This unusual effect could not arise simply from the changed environment of the optically active centers. In another experiment it was shown that the racemic polypeptide formed by using L-polypeptide to initiate the polymerization of a D,L N-carboxyanhydride was itself optically active.[3] This showed clearly the preservation of at least some of the screw sense characteristic of the L-polypeptide in the grafted racemic polypeptide and the association of optical activity with the helical configuration. In a third experiment the optical rotation of a series of copolymers of D- and L-residues was shown to increase linearly as the concentration of D-residues increased from zero to 10–30 per cent.[4, 5] Extrapolation from this linear region to 50 per cent D-residues yielded a positive value which was an estimate of the optical rotation of a helix composed of racemic residues. As a consequence of these experiments, there remains no doubt that the difference in rotation between the helical and randomly

coiled forms is due *primarily* to the contribution to the optical rotation of the helical configuration itself, a contribution that has only one screw sense, presumably right-handed.[6-9]

The observed difference in rotation exhibited by the two configurations does not, however, measure the contribution of the helical configuration to the optical rotation, because the intrinsic residue rotations may be quite different in one configuration than in the other, and the solvent effect alters the rotation, particularly in the randomly coiled form, to a substantial and unpredictable extent. The purpose of this communication is to establish precisely the rotatory dispersion of a racemic polypeptide in the α-helical configuration having a single screw sense, that is, of a polypeptide whose optical activity is derived only from its helical configuration.

From previous studies of rotatory dispersion of polypeptides of optically active amino acids,[2, 7, 10, 11] it is clear that both a normal and an anomalous dispersive term are required to represent the data when a substantial amount of the helical form is present. These two terms are shown in the following equation, which provides a close fit for all data thus far obtained.[6]

$$[m'] = \frac{3}{n^2+2}[m] = \frac{3}{n^2+2}\frac{M_0}{100}[\alpha] = \frac{a_0\lambda_0^2}{(\lambda^2-\lambda_0^2)} + \frac{b_0\lambda_0^4}{(\lambda^2-\lambda_0^2)^2},$$

where $[m']$ is the *effective residue rotation* (independent of refractive index of the solution, n); $[m]$ is the residue rotation (M_0 being the molecular weight of the monomer unit); and $[\alpha]$ is the *specific rotation* defined in the usual way. Thus the evaluation of three constants, a_0, b_0, and λ_0, is required for the characterization of the rotatory dispersion.

From theoretical investigation of the optical properties of helical macromolecules, it appears that the first term, the normal dispersive term, may have at least two components: one or more arising from the intrinsic residue rotation and the other from the helical configuration. The second term, the anomalous dispersive term, arises only from the helical configuration.[6] Thus, if the rotatory dispersion of a racemic helix having a single screw sense could be obtained, a_0 and b_0 would have unambiguous meanings and, moreover, the intrinsic residue rotation of optically active residues in the helical configuration could be obtained from the difference between the value of a_0 for the racemic case and the value obtained from the rotatory dispersion of the enantiomorph. Perhaps the most useful application of such a result would be in the quantitative basis it would provide for the determination of the fraction of residues that exist in the helical configuration in protein molecules.[10]

The Preparation of Appropriate Helical Polypeptides.—If a polypeptide is prepared from a racemic N-carboxyanhydride by the usual polymerization procedure (initiation with an aliphatic amine), the product is, of course, optically inactive. However, if polymerization is initiated with a preformed polymer composed of L-residues and having a helical configuration, the racemic polymer formed is optically active, as shown from the increase in optical rotation accompanying the polymerization.[3] The optical activity of the racemic polymer in this case arises from the preferential screw sense imposed on the polymer by the initiating L-polypeptide. Further consideration, however, shows that it is unlikely that the screw sense of the initiating polymer has been completely preserved in the racemic polymer, hereafter

referred to as the *grafted polymer*. This is evident from the fact that the specific rotation of the grafted polymer diminishes with its length (i.e., molecular weight) and the observation that, when the polymerization of D-anhydride is initiated with L-polypeptide, the screw sense of the initiator is preserved only for about four residues, following which there is an inversion of the helix of the grafted polymer to the opposite screw sense. This second observation provides an explanation of the first. If the occurrence of a sequence of four or more enantiomorphic residues causes helix inversion, it follows that inversions can occur in grafted polymer made from the racemic anhydride and that the frequency of inversion will increase with chain length to the point where the grafted polymer will have lost completely the screw-sense preference induced by the initiator and will then approach optical inactivity.

As a consequence of these considerations, it appears that the screw sense of the initiating polymer could be preserved only by diminishing the concentration of D-anhydride relative to L-anhydride to the point where the probability of occurrence of quartets and longer sequences of D-residues within the chain length of the grafted polymer becomes negligible. Then, by subtracting the rotation due to the excess L-residues from the observed rotation of the polymer, the rotation due to the racemic residues in a helix of a single screw sense would be obtained. This is the procedure we have followed.

The initiating polypeptide was made by polymerizing the N-carboxyanhydride of γ-benzyl-L-glutamate in dimethylformamide solution (4 per cent) at an anhydride-initiator ratio of 15. This value was chosen because it is the lowest for which the polymer will be practically all in the helical configuration in a solvent favorable to this configuration. Following isolation of the polymer, aliquots of it were used as initiator for anhydride of the same composition but of various D-L ratios. These polymerizations were carried out at 25° C. in freshly purified dioxane containing 1.75 gm/100 cc of initiating polymer and sufficient anhydride to increase the degree of polymerization of each initiating molecule by 25. In each case the polymerization was allowed to proceed for at least 50 hours to insure completeness of the reaction.

The product of each reaction was itself a solution of the desired polypeptide in dioxane at 4.05 gm/100 cc concentration. This was transferred to a 2-decimeter polarimeter tube, and its optical rotation at twelve wave lengths between 350 and 750 mμ was measured directly in a Rudolph spectropolarimeter. The results are listed in Table 1, where f_D denotes the fraction of residues of the D-configuration in each grafted polymer. The reproducibility of these values fell within the range listed in the second column. The observed rotations, converted to specific rotation by multiplication by (100/8.1), are plotted in Figure 1.

The specific rotation of the D-residues and an equivalent number of L-residues for each grafted polymer were obtained in the following way. The rotation of the initiating polypeptide was considered constant and subtracted from the observed rotation. In addition, a subtraction was made for the unmatched L-residues contained in the grafted polymer. This quantity was computed by multiplying the difference between the rotation of the pure L-polypeptide (next to last column) and the initiating polypeptide (last column) by the fraction of residues that were uncompensated. The specific rotation was then computed in the usual manner.

The calculation is summarized as follows:

$$[\alpha]^{DL} = \frac{100}{lc}\,\alpha^{DL} = \frac{100}{(2)(2.30)(2f_D)}\,[\alpha_{obs} - \alpha_{init} - (1 - 2f_D)(\alpha_L - \alpha_{init})].$$

TABLE 1

OBSERVED ROTATIONS OF D,L-COPOLYMERS OF POLY-γ-BENZYL-L-GLUTAMATE INITIATED BY L-POLYMER

λ	EXPTL. ERROR	f_D OF GRAFTED POLYMER 0.50	0.40	0.35	0.30	0.20	0.10	0	INITIATING L-POLYMER
3500	±0.04°	1.43°	2.91°	2.40°	1.66°	−0.23°	−2.28°	−4.08°	−0.72°
3750	± .04	2.03	3.53	3.19	2.72	1.21	−0.36	−1.74	− .09
4000	± .03	2.17	3.64	3.42	3.04	1.83	0.56	−0.55	.21
4250	± .03	...	...	3.37	...	2.08	1.02	0.09	.36
4500	± .02	2.06	3.34	3.20	2.99	2.13	1.24	0.45	.43
5000	± .02	1.80	2.87	2.82	2.65	2.04	1.34	0.76	.47
5500	± .01	1.56	2.45	2.42	2.29	1.84	1.27	0.82	.44
5890	± .01	1.38	2.17	2.16	2.05	1.64	1.20	0.81	.41
6000	± .01	1.34	2.12	2.11	1.99	1.59	1.18	0.81	.40
6500	± .02	1.17	1.83	1.82	1.73	1.39	1.06	0.80	.36
7000	± .02	1.02	1.60	1.60	1.50	1.21	0.98	0.71	.33
7500	±0.03	0.92	1.42	1.46	1.35	1.12	0.88	0.64	0.32

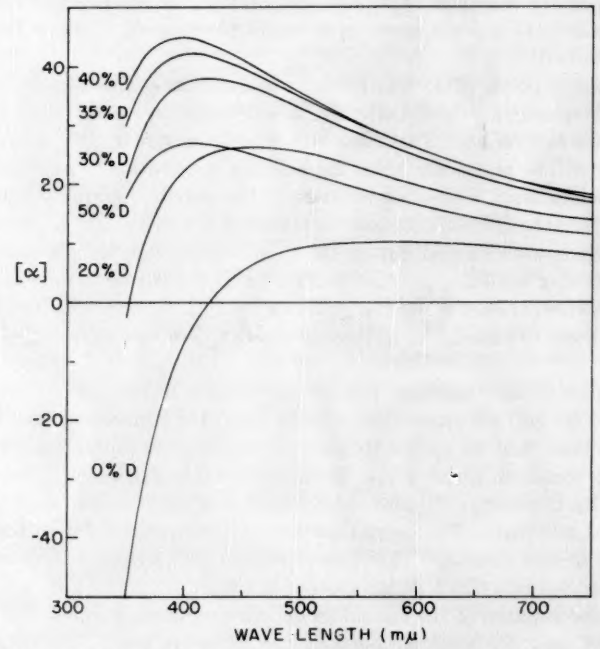


FIG. 1.—Rotatory dispersion of polypeptide chains containing various proportions of D- and L-residues (γ-benzyl glutamate). Solvent: dioxane. Figures denote composition of grafted portion of polypeptide chain.

The Optical Rotation (Sodium D Line) of a Racemic α-Helix.—The values of the specific rotation of the racemic portion of the grafted polymer, $[\alpha]^{DL}$, are plotted in Figure 2 for the wave length 5890 A, the sodium D line. It is seen that the value

for 50 per cent D-residues in the grafted polymer is +21° and that the value increases as the fraction of D-residues decreases, reaching a constant value of +55° below 30 per cent. This is the behavior that was anticipated. That is, as the D-residue concentration is diminished, the frequency of helix inversion falls rapidly to zero, leaving the screw sense of the initiating helix unaltered throughout the entire polypeptide. Since the relative importance of experimental errors increases as the composition approaches pure L-polymer, the result at 10 per cent D-residues has a large uncertainty. We conclude, therefore, that the value of 55° given by the polymer having a composition of 20 per cent D (and another polymer, not listed in the table, which had a composition of 19 per cent) represents the desired quantity. A review of possible errors suggests that the uncertainty of this figure is about ±2°.

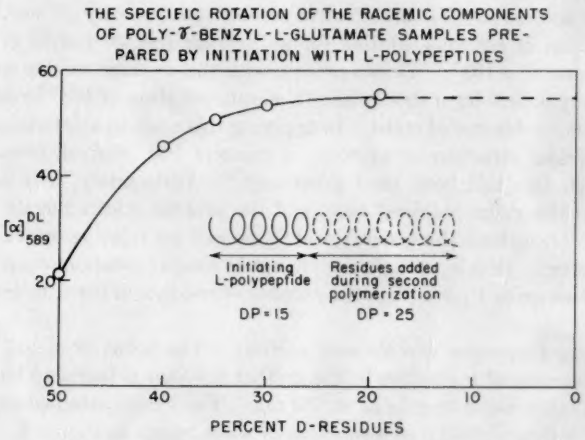


FIG. 2.—Specific rotation of the racemic components of poly-γ-benzyl glutamate samples prepared by initiation with L-polypeptides. Solvent: dioxane.

Before discussing this result, it is perhaps of interest to note that the value of $[\alpha]^{DL}$ for the 50 per cent D-residue case (i.e., +21°) is indeed close to the value that would be estimated on the basis of inversion of the helix occurring when sequences of four or more D-residues occur in a helix whose screw sense is that of the L-polypeptide. By direct iteration we have counted the probability of the occurrence of a quartet of D-residues and tabulated the number of residues that might be located in the inverted helix if the inversion were brought about by the random occurrence of quartet of D-residues in the growth of the grafted polymer. This result has been corrected for the frequency of reinversion due to a later occurrence of a quartet of L-residues following a quartet of D-residues. On this basis we find that 29 per cent of the residues occurring in the grafted polymer of degree of polymerization 25 should exist in inverted helices, that is, the helix characteristic of the D-residues. Since this fraction will contribute a specific rotation of −55° to $[\alpha]^{DL}$, it follows that the expected value of $[\alpha]^{DL}$ is +23°. The agreement between this result and the observed value is as close as the crudeness of our model

would lead us to expect, and it confirms the description of the polymerization of enantiomorphs deduced above.

Upon converting this value for the specific rotation of a racemic α-helix of poly-γ-benzyl-glutamate to the effective residue rotation, $[m']^{DL}$, a result is obtained that is independent of refractive index and residue weight. The result is: $[m']_D^{DL} = 90°$. Previous estimates of this quantity by extrapolating rotation measurements on D,L-copolymers led to 110° for leucine[4] and 78° for poly-γ-benzyl-L-glutamate.[5] Before these particular estimates had been made, there was a tendency to use the difference in specific rotation between the random coil and helical configurations for estimating this quantity. Taking into account all the data now available in various solvents,[10] the values obtained in this way range from 40° to 145°. Thus the value that we have determined lies near the center of the previous wide range of estimated values.

For use in protein studies this effective residue rotation may be converted to the specific rotation in aqueous solution for an average residue weight of 110. This leads to a value of +103°. Thus a protein with this average residue weight and in the completely helical form should have a specific rotation of 103° in excess of that of the completely denatured state. In applying this scale to a determination of the amount of helical structure in proteins, a value of 100° derived from poly-L-glutamic acid studies had been used previously.[10] Fortunately, this estimate was very close to the value obtained here, and the protein results remain unchanged. Indeed, this strengthens the general guide proposed for relating optical rotation to protein structure: that is, each degree change in specific rotation of a protein corresponds to a change of 1 per cent in the number of residues in the α-helical configuration.[10]

The Rotatory Dispersion of a Racemic α-Helix.—The behavior of $[\alpha]^{DL}$ as a function of the fraction of D-residues in the grafted polymer is found to have the same character at other wave lengths as at 589 mμ. The values attained at the plateau ($f_D = 0.30 - 0$) are plotted as a function of wave length in Figure 3. This represents the rotatory dispersion of an α-helix arising from its configuration alone. The helix sense is, of course, that characteristic of L-polypeptides.

The rotatory dispersion shown in Figure 3 can be fitted with the two-term equation given in the introduction. If λ_0 is assigned the previously established value of 2120 A, a plot of $[\alpha](\lambda^2 - \lambda_0^2)$ against $(\lambda^2 - \lambda_0^2)^{-1}$ gives a linear plot from which the values of the other two constants are readily obtained: $a_0 = 680°$ and $b_0 = -500°$. The curve in Figure 3 is a plot of the two-term equation with these values of the three constants. It is important to note, however, that our data permit considerable latitude in choosing the value of λ_0. Indeed, the linearity of the foregoing plot would be maintained for any value of λ_0 within 10 per cent of 2120 A.[12] Since the values assigned to a_0 and b_0 depend strongly on the choice of λ_0, the values given here must be associated with the value of 2120 A for λ_0.

The value of b_0 previously available was obtained from dispersion data on poly-γ-benzyl-L-glutamate and poly-L-glutamic acid: the values ranged from −580° to −660°, with an average of −625°.[7] The difference between this and the value obtained in this study (−500°) lies somewhat outside probable experimental error, but at least two explanations can be put forward. One is experimental in origin. The rotation of the initiating polymer was a little different (particularly at low

wave lengths) than that of higher-molecular-weight samples of the same substance under identical conditions. This was probably due to the initiator containing a small amount of polymer so low in molecular weight that it was not helical. The rotational contribution of this small fraction of material would, however, change during formation of the grafted polymer, since it would then become helical. The maximum effect of this contribution would be an increase in the value of b_0 to $-580°$, but we consider the actual effect to be much less. On the theoretical side, it must be pointed out that several normal terms may exist with similar but not identical values of λ_0 in the case of L-polypeptides. The effect produced when these are treated as a single term may lead to a bias on the value of λ_0 associated with the

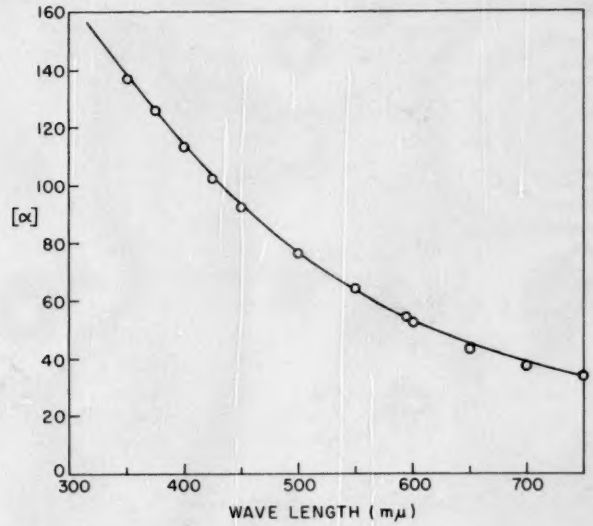


FIG. 3.—The rotatory dispersion of the racemic helix of poly-γ-benzyl-DL-glutamate in the form of an α-helix having a single screw sense (probably right-handed). The line is a plot of the two-term equation with $a_0 = 680°$, $b_0 = -500°$, and $\lambda_0 = 2120$ A.

helical configuration alone. A shift in the assigned value of λ_0 would, of course, alter the assigned value of b_0. Moreover, there is also the possibility that in L-polypeptides the side chains are sufficiently oriented in the helical framework that their chromophoric groups may make a small contribution to the over-all rotation. There is, therefore, no reason to expect better agreement than that which has been found.

Three observations may be made from the determination of the rotatory dispersion arising from the helical configuration alone. The first is that the form of the dispersion is that of the two-term equation in agreement with theoretical considerations.[6, 8] Since the value of b_0 is found to be close to that found for L-polypeptides, it is clear that the side chains have little effect on the rotational contribution made by the helical configuration.

The other two observations make use of the value of the helical contribution to a_0 now available for the first time. Considering first only the sodium D line values, it is seen that the effective residue rotation due to the helical configuration alone, and therefore more properly denoted with a superscript H than by the superscript DL, is made up of contributions from the two terms as follows:

$$[m']^{H}_{D} = 101 - 11 = 90°.$$

This shows clearly the relative importance of the normal and anomalous terms at this wave length: the normal term is much the larger and dictates the sign of $[m']^{H}_{D}$.

When L-polypeptides and proteins are in the helical configurations, the second term will remain essentially unchanged, but the first term will be altered by the addition of the intrinsic residue rotations *in the helical configuration*. This latter qualification is necessary because the environment of the asymmetric center in the helical configuration will be expected to produce its own "solvent effect." For example, $[m']_D$ for poly-γ-benzyl-L-glutamate is observed to be 16.3° in dioxane. This means that the contribution of the intrinsic residue rotations is −73.7°, and the observed value can be thought to be made up as follows:

$$[m']_D = (101 - 73.7) - 11 = 27.3 - 11 = 16.3.$$

Most L-residues appear to behave in a similar manner: that is, they have an intrinsic residue rotation in range of −90 to −70° which nearly cancels the value of the normal dispersive term arising from the helical contribution. The result of this is to bring the relative importance of the two terms much closer together, producing a small and generally positive value for $[m']_D$ itself. Reverting to aqueous solutions of proteins with an average residue weight of 110, this would mean that in the completely helical form the value of $[\alpha]_D$ should be in the range of 0°–23°.

The rotation of L-polypeptides and proteins in solutions where they exist in randomly coiled configurations can now be viewed in better perspective. Thus $[m']_D$ has been found to have the following values for poly-γ-benzyl-L-glutamate in dichloracetic acid, hydrazine, and trifluoracetic acid, respectively:[10] −26.5°, −52.5°, and −75.5°. Since only the last of these values is close to −73.7°, it appears that the strongest solvating solvent, trifluoroacetic acid, is required to match the solvent effect, that is, the effective environment, that the residues experience in the helical configuration. In aqueous solutions of polypeptides and proteins, however, the energy differences between helical and randomly coiled configurations are much less, and as a consequence the solvent effects on rotation are expected to be much less. This is generally borne out in the preliminary studies that have been made.[10]

The final observation concerns the application of these same considerations to the rotatory dispersion rather than only to the sodium D line rotations. For the two extremes of the wave-length scale employed in this work the contributions of the helical configuration to the two terms are seen to be as follows:

$$[m']^{H}_{350.} = 394 - 167 = 227°;$$

$$[m']^{H}_{750.} = 59.1 - 3.8 = 55.3°.$$

It can be seen from these examples how the relative importance of the anomalous term increases with decreasing wave length.[13] The intrinsic residue rotations of L-polypeptides and proteins become increasingly negative with decreasing wave length, so much so that below a certain wave length this dominates the normal term and the values of $[m']$ pass through zero and reach quite negative values.

Now that the rotatory dispersion due to the helical configuration is available, it can be used in conjunction with the rotatory dispersion of completely helical L-polypeptides or proteins to provide the rotatory dispersion of the residues (asymmetric centers) as they exist in the helix. This should be particularly useful in the quantitative study of protein structure and denaturation because the criterion is then available by which a denaturing solvent can be chosen in which the solvent effect is equivalent to that which the residues experience in the helix. Changes in rotation studied in such a system would then very accurately reflect the amount of helical configuration present, and a search could be undertaken for minor structural features that might also influence the optical rotation through configurations other than the right-handed α-helix.

Summary.—The evidence showing that L-polypeptides can exist in solution as α-helices having a single screw sense, probably right-handed, is reviewed. It is then shown that the rotatory properties of such a helix devoid of the effects of asymmetric centers can be determined from properly prepared polypeptides. These polypeptides were made by using preformed L-polypeptides having the desired screw sense to initiate the polymerization of mixtures of D- and L-anhydrides. From rotatory dispersion measurements on these it is possible to show that the helical configuration is responsible for the following effective residue rotation, $[m']^H$:

$$[m']^H = \frac{680\lambda_0^2}{(\lambda^2 - \lambda_0^2)} - \frac{500\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} = \frac{3}{n^2 + 2}\frac{M_0}{100}[\alpha]^H,$$

where M_0 = residue molecular weight; n = refractive index of solvent; λ = wave length; λ_0 = 2120 A; and $[\alpha]^H$ = specific rotation. For the sodium D line, $[m']_D^H = +90°$, and for a protein in aqueous solution having an average residue weight of 110, $[\alpha]_D^H = +103°$. The implications of these results are discussed.

We wish to express our appreciation to Dr. J. T. Yang for his aid in the planning and execution of this work and to Dr. E. R. Blout for providing the N-carboxyanhydrides and much helpful advice.

* This investigation was supported in part by the Office of Naval Research (N5ori-07654). This paper is No. XIV in a series of which the last was by E. R. Blout and H. Lenermant, submitted to *Nature*.

† Public Health Service Research Fellow, 1955–1957.

[1] P. Doty, A. M. Holtzer, J. H. Bradbury, and E. R. Blout, *J. Am. Chem. Soc.*, **76**, 4493, 1954; P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947, 1956.

[2] P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **78**, 498, 1956.

[3] P. Doty and R. D. Lundberg, *J. Am. Chem. Soc.*, **78**, 4810, 1956.

[4] A. Elliott, W. E. Hanby, and B. R. Malcolm, *Nature*, **178**, 1170, 1956.

[5] E. R. Blout, P. Doty, and J. T. Yang, *J. Am. Chem. Soc.*, in press.

[6] W. Moffitt, *J. Chem. Phys.*, **25**, 467, 1956.

[7] W. Moffitt and J. T. Yang, these PROCEEDINGS, **42**, 596, 1956.

[8] D. D. Fitts and J. G. Kirkwood, *J. Am. Chem. Soc.*, **78**, 2650, 1956.

[9] A. Elliott and B. R. Malcolm, *Nature*, **178**, 912, 1956.

[10] J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, in press.

[11] C. Cohen and Andrew G. Szent-Gyorgyi, *J. Am. Chem. Soc.*, **79**, 248, 1957.

[12] The observed values recorded in the table when plotted as $[\alpha](\lambda^2 - \lambda_0^2)$ against $(\lambda^2 - \lambda_0^2)^{-1}$ fit a straight line much more closely than do the points in Figure 3. The scatter in the plot of the latter and most of the uncertainty in the values of λ_0 and b_0 come from the increase in percentage error introduced by taking differences. In addition, the anomalous term is very small relative to the normal term for the racemic case. In L-polypeptides this is not the case, and as a consequence λ_0 can be determined more precisely.

[13] It is of interest to note, however, that the extrapolation of Moffitt's equation, with the values assigned to a_0, b_0, and λ_0, to lower wave lengths shows $[m']^H$ to pass through a maximum and to become negative below 2750 A.

CONTRIBUTIONS TO THE THEORY OF THE CONTINUOUS BACTERIAL GROWTH APPARATUS

*I. KINETICS OF GROWTH OF HOMOGENEOUS POPULATIONS**

By H. Moser

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR, NEW YORK

Communicated by B. P. Kaufmann, December 19, 1956

The theories of the bacterial continuous growth apparatus presented by Monod,[1] Novick and Szilard,[2] Spicer,[3] and Herbert *et al.*[4] furnish equations which describe the steady state of homogeneous bacterial populations in culture devices such as the chemostat. They do not, however, provide mathematical proof of the automatic establishment of the steady state when for example, one starts at the time of inoculation with a nonsteady state.

If c stands for the concentration of limiting growth factor (LGF) in the culture vessel, k for the growth-rate constant (specific growth rate[4]), v for the inviability constant, ω for the washout-rate constant (flow rate per unit culture volume), a for the concentration of LGF in the incoming medium, q for the uptake of LGF per bacterium produced (assimilation coefficient, yield coefficient),[4, 5] and N for the population density, the differential equations for the rate of change of cell density and of concentration of LGF in the culture vessel of the chemostat are

$$\frac{1}{N}\frac{dN}{dt} = k(c) - v(c) - \omega \qquad (1a)$$

and

$$\frac{dc}{dt} = \omega(a - c) - qk(c)N, \qquad (2a)$$

respectively.

The specific growth rate is a nonlinear function of c; it approaches a maximum value when $c \to \infty$.[2, 6] Up to a certain value of c, however, the specific growth rate approximates a linear function of c. Within the range in which specific growth rate is approximately proportional to c, production of inviable cells is negligible. Thus, if c is kept sufficiently low by starting with a low initial concen-

tration, c_0, and by maintaining an adequate flow rate, we can set for the specific growth rate and the viability constant $k(c) = \kappa c$ and $v(c) = 0$, respectively (κ = factor of proportionality), and equation (1*a*) and (2*a*) are reduced to

$$\frac{dN}{dt} = (\kappa c - \omega)N \tag{1b}$$

and

$$\frac{dc}{dt} = \omega(a - c) - q\kappa cN, \tag{2b}$$

respectively. The physical nature of the problem demands that $N_0 > 0$ and $a\kappa > \omega$. The concentrations of cells ($\tilde{N}$) and of LGF ($\tilde{c}$) at equilibrium are found by equating the derivatives to zero:

$$\tilde{c} = \frac{\omega}{\kappa}, \tag{3}$$

$$\tilde{N} = \frac{a - \tilde{c}}{q}. \tag{4}$$

A solution of the two simultaneous differential equations (1*b*) and (2*b*) can be given in convenient terms.

Multiplication of equation (1*b*) by q and addition of equation (2*b*) result in the equation

$$\frac{d}{dt}(qN + c) + \omega(qN + c) - a\omega = 0 \tag{5}$$

for the quantity $qN + c$. Integration of equation (5) and determination of the integration constant with the initial condition $qN_0 + c_0$ at $t = t_0 = 0$ furnishes for c the expression

$$c = a + (c_0 + qN_0 - a) \exp(-\omega t) - qN. \tag{6}$$

If for convenience we set $A = \kappa a - \omega$, $B = -(\kappa/\omega)(c_0 + qN_0 - a)$ and substitute expression (6) for c in equation (1*b*), we obtain for the cell density the differential equation

$$\frac{dN}{dt} - (A - B\omega \exp(-\omega t))N + \kappa qN^2 = 0. \tag{7}$$

The theory of Bernoulli's differential equation furnishes for N the expression

$$1/N(t) = \exp(-At - Be^{-\omega t})\left(C + \kappa q \int^t \exp(At + Be^{-\omega t})dt\right). \tag{8}$$

When the integration constant C is evaluated with the initial condition $N(0) = N_0$, the solution of (1*b*) and (2*b*) for N is

$$N(t) = \frac{N_0 \exp(At + Be^{-\omega t})}{\exp(B) + \kappa qN_0 \int_0^t \exp(At + Be^{-\omega t})dt} \tag{8a}$$

For large t the factor $Be^{-\omega t}$ tends toward zero, and the remaining integral in (8a) behaves like

$$\int_0^t \exp(At)dt = (1/A)(\exp(At) - 1). \tag{9}$$

Insertion of (9) into (8a) gives for large t

$$N(t) = \frac{\exp(Be^{-\omega t})}{((e^B/N_0) - (\kappa q/A))\, e^{-At} + (\kappa q/A)} \tag{10}$$

From (10) we derive

$$\lim_{t \to \infty} N(t) = A/\kappa q = \tilde{N},$$

and subsequently from (6)

$$\lim_{t \to \infty} c(t) = a - q \cdot \lim_{t \to \infty} N(t) = \omega/\kappa = \tilde{c}.$$

Thus we have proved analytically that a stable stationary state establishes itself in a homogeneous bacterial population growing in the chemostat.

A satisfactory approximation to the solution of (1b) and (2b) for N can be derived from (8a). When we write the remaining integral in (8a) in the form

$$\int_0^t \exp(Ax + Be^{-\omega x})dx$$

and substitute B^1/ω for the constant $B = (-\kappa/\omega)(c_0 + qN_0 - a)$ we obtain for N the expression

$$N(t) = \frac{N_0 \exp\left(At - B'\left[\dfrac{1 - e^{-\omega t}}{\omega}\right]\right)}{1 + \kappa q N_0 \displaystyle\int_0^t \exp\left(Ax - B'\left[\frac{1 - e^{-\omega x}}{\omega}\right]\right) dx} \tag{11}$$

or

$$N(t) = \frac{N_0 \exp\left(At - B'\left[\dfrac{e^{\omega t} - 1}{e^{\omega t}}\right]\right)}{1 + \kappa q N_0 \displaystyle\int_0^t \exp\left(Ax - B'\left[\frac{e^{\omega t} - e^{\omega(t-x)}}{\omega e^{\omega t}}\right]\right) dx}. \tag{12}$$

A satisfactory estimate of the remaining integral in (12) is

$$\int_0^t \exp\left(Ax - B'\left[\frac{x}{1 + \omega t}\right]\right) dx = \frac{1 + \omega t}{A(1 + \omega t) - B'}\left\{\exp\left[\frac{A(1 + \omega t) - B'}{1 + \omega t} \cdot t\right] - 1\right\};$$

a corresponding estimate of the numerator of (12) is

$$N_0 \exp\left(\frac{A(1 + \omega t) - B'}{1 + \omega t} \cdot t\right).$$

Then the resulting approximation for the cell density can be written in the form

$$N(t) = \frac{\epsilon(t)}{\kappa q + \left(\frac{\epsilon(t)}{N_0} - \kappa q\right) \exp\left(-t\epsilon(t)\right)} \tag{14}$$

in which $\epsilon(t) = \dfrac{A(1+\omega t) - B'}{1+\omega t} = \dfrac{\kappa(qN_0 + c_0) - \omega + \kappa q\omega\tilde{N}t}{1+\omega t}$.

This approximation to the solution of (1*b*) and (2*b*) for N describes the automatic adjustment of growth equilibrium in the chemostat as a function of time. It furnishes the correct initial density, $N(0) = N_0$, the true initial derivative $(dN/dt)_{t=0} = (\kappa c_0 - \omega)N_0$, and the true equilibrium value, $\tilde{N}$, for $t = \infty$. Since measurement of bacterial density is easy and fairly accurate, equation (14) provides a convenient tool for testing the theory of the continuous bacterial growth apparatus under a variety of given experimental conditions.

Thus we have proved analytically that a stable stationary state establishes itself in a homogeneous bacterial population growing in the chemostat, provided, of course, that $a > \omega/\kappa$.

Equations (10), (10*a*), and (11) describe the automatic adjustment of growth equilibrium in the chemostat as a function of time, but for numerical analysis and curve-fitting these equations are inconvenient. It is possible, however to derive a satisfactory approximation for $N(t)$ which facilitates computation. Equation (8) can be written in the form

$$\frac{1}{N(t)} = \exp\left(-At - Be^{-\omega t}\right)\left(C + \kappa q \int^t \exp\left(Ax + Be^{-\omega x}\right) dx\right); \tag{8a}$$

or, when C is evaluated with the initial condition $N(0) = N_0$ and B'/ω is substituted for $B = (-\kappa/\omega)(c_0 + qN_0 - a)$, it may be written either as

$$N(t) = \frac{N_0 \exp\left(At - B'[(1 - e^{-\omega t})/\omega]\right)}{1 + \kappa q N_0 \int_0^t \exp\left(Ax - B'[(1 - e^{-\omega x})/\omega]\right)dx} \tag{8b}$$

or as

$$N(t) = \frac{N_0 \exp\left(At - B'[(e^{\omega t} - 1)/e^{\omega t}]\right)}{1 + \kappa q N_0 \int_0^t \exp\left(Ax - B'[(e^{\omega t} - e^{\omega(t-x)})/\omega e^{\omega t}]\right)dx}. \tag{8c}$$

A satisfactory estimate of the remaining integral in expression (8*c*) is

$$\int_0^t \exp\left(Ax - B'\left[\frac{x}{1+\omega t}\right]\right) dx = \frac{1+\omega t}{A(1+\omega t) - B'}\left\{\exp\left[\frac{A(1+\omega t) - B'}{1+\omega t} \cdot t\right] - 1\right\}$$

a corresponding estimate of the numerator of expressions (8*c*) is

$$N_0 \exp\left(\frac{A(1+\omega t) - B'}{1+\omega t} \cdot t\right).$$

Then the resulting approximation for the cell density can be written in the form

$$N(t) = \frac{\epsilon(t)}{\kappa q + (\epsilon(t)/N_0) - \kappa q) \exp(-t\epsilon(t))}, \quad (14)$$

in which

$$\epsilon(t) = \frac{A(1 + \omega t) - B'}{1 + \omega t} = \frac{\kappa(qN_0 + c_0) - \omega + \kappa q \omega \bar{N} t}{1 + \omega t}.$$

This approximation to the solution of equation (7) furnishes the correct initial density, $N(0) = N_0$, the true initial derivative $(dN/dt)_{t=0} = (\kappa c_0 - \omega)N_0$, and the true equilibrium value, $\bar{N}$, for $t = \infty$. Since measurement of bacterial density is easy and fairly accurate, equation (14) provides a convenient tool for testing the theory of the continuous bacterial growth apparatus under a variety of given experimental conditions.

* Preparation of this paper was begun while the author held a fellowship of the Carnegie Institution of Washington at the Department of Genetics, and it was completed at the Biological Laboratory of the Long Island Biological Association while the author engaged in a project sponsored by the United States Atomic Energy Commission under Contract AT(30-1)-1944.

The author wishes to express his sincere gratitude to Dr. F. Joachim Weyl, of the Department of the Navy, Office of Naval Research, Washington, D.C., for suggesting this approximation for $N(t)$ (eq. [14]), and to Dr. M. Delbruck of the California Institute of Technology, for helpful comments.

[1] J. Monod, "La Technique de culture continue," *Ann. Inst. Pasteur*, **79**, 390–410, 1950.

[2] A. Novick and L. Szilard, "Experiments with the Chemostat on Spontaneous Mutations of Bacteria," these PROCEEDINGS, **36**, 708–719, 1950.

[3] C. C. Spicer, "The Theory of Bacterial Constant Growth Apparatus," *Biometrics*, **11**, 225–230, 1955.

[4] D. Herbert, R. Elsworth, and R. C. Telling, "The Continuous Culture of Bacteria: A Theoretical and Experimental Study," *J. Gen. Microbiol.*, **14**, 601–622, 1956.

[5] H. Moser, "The Dynamics of Bacterial Populations Maintained in the Chemostat," *Carnegie Inst. Washington Publ.* (in press).

[6] J. Monod, *La Croissance des cultures bactériennes* (Paris: Hermann & Cie, 1942).

HETEROSIS AND ELIMINATION OF WEAK HOMOZYGOTES IN NATURAL POPULATIONS OF THREE RELATED SPECIES OF DROSOPHILA

BY CRODOWALDO PAVAN, THEODOSIUS DOBZHANSKY,* AND ANTONIO BRITO DA CUNHA

DEPARTAMENTO DE BIOLOGIA, FACULDADE DE FILOSOFIA, SÃO PAULO, BRAZIL, AND DEPARTMENT OF ZOOLOGY, COLUMBIA UNIVERSITY, NEW YORK

Large stores of genic and chromosomal variants are carried in the Mendelian populations of sexually reproducing and cross-fertilizing species. These variants arise ultimately by mutation, but mutant genes and chromosomes may be retained in the gene pool of a population for many generations after their origin. Opportunity is thus provided for manipulation of the store of mutants in the gene pool of

the population by natural or artificial selection. The trend of selection is, of course, toward making the greatest possible number of the available genetic variants serve the adaptive needs of the species. However, most mutations that arise are deleterious to their carriers, at least in the environments in which the species has been formed and in which it normally lives. Mutation and selection are conflicting forces: the former generates injurious genetic variants which constitute "our load of mutations";[1, 2] the latter tends to utilize a minority of the mutants which are capable of being turned to some advantage. Therefore, it may be grossly misleading to think that newly arising mutants merely add an increment qualitatively similar to the already existing load. This is especially so where mutants induced by high-energy radiations are concerned. The scene is too diverse and contradictory to make such an assumption safe. The evidence available is clearly inadequate. Relatively much more is known about the properties of newly arisen mutants, whether spontaneous or induced by radiation, than about those which persist in the populations as components of the mutational load. Some of the latter exhibit rather startling properties, for which an acquaintance with newly induced mutants leaves one insufficiently prepared. The chief among these properties is the ability of some naturally occurring genetic variants to engender hybrid vigor in heterozygotes and yet to reduce, often drastically, the fitness of the homozygotes. The possibility must be seriously considered that the injurious effects of radiation-induced and of freshly arisen spontaneous mutants may be on the average greater than those of the variants carried in the populations. The purpose of the present article is to record some instances of naturally occurring variants which are heterotic when heterozygous and deleterious when homozygous.

Caribbean Populations of Drosophila tropicalis.—One of us[3] has reported the findings in a remarkable population of *D. tropicalis* collected at Lancetilla, Honduras, in April, 1954, by Dr. William B. Heed. In the progeny of 74 wild females tested, 70.3 per cent of the larvae were found to be heterozygous for a certain subbasal inversion in the left limb of the second chromosome. The 74 strains derived from these females served as progenitors of an experimental population; after breeding in the laboratory for about 4 months, 90.2 per cent of the larvae obtained from the eggs deposited in this population were heterozygous for the same inversions, and 96.0 per cent of the adults which developed in the experimental population were heterozygous. The adaptive norm in this population consisted of individuals heterozygous for the inversion, while the corresponding homozygotes were subvital or semilethal.

Owing to the courtesy of Drs. W. B. Heed, M. R. Wheeler, W. S. Stone, and H. L. Carson, we were privileged to examine samples of the populations of *D. tropicalis* collected (chiefly in 1956) in ten groups of localities in Central America, the West Indies, and Colombia, South America. The proportions of the larvae found to be heterozygous for the subbasal inversion in the II L chromosome are as shown in Table I (one larva examined in the progeny of each female collected in nature). The results are mapped in Figure 1, the heights of the black columns symbolizing the percentages of the heterozygotes for the subbasal inversion in chromosome II L (a scale for the percentages is shown in the upper right corner of the map). This inversion occurs, then, in a territory extending from the Island of Hispaniola to Honduras, San Salvador, and Costa Rica. Although the samples

TABLE 1

No.	Locality	No. Examined	Per Cent Heterozygotes
1*A*	San Salvador (1954)*	23	34.8
1*B*	San Salvador (1955)	10	40.0
2	Lancetilla, Honduras*	74	70.3
3	Bath, Jamaica	38	11.5
4	Santiago de Cuba	10	20.0
5*a*	Petionville, Haiti	10	40.0
5*b*	Dominican Republic†	10	40.0
6	El Yunque National Forest, Puerto Rico	8	0
7	Santa Marta, Colombia	18	0
8	Bucaramanga, Colombia	2	0
9	Barro Colorado, Panama (1955 and 1956)	48	0
10*A*	Turrialba, Costa Rica	10	0
10*B*	La Lola, Costa Rica	26	3.8

* Th. Dobzhansky and O. Pavlovsky, these PROCEEDINGS, **41**, 289–295, 1955.
† J. I. Townsend, *Am. Naturalist*, **88**, 339–351, 1954.

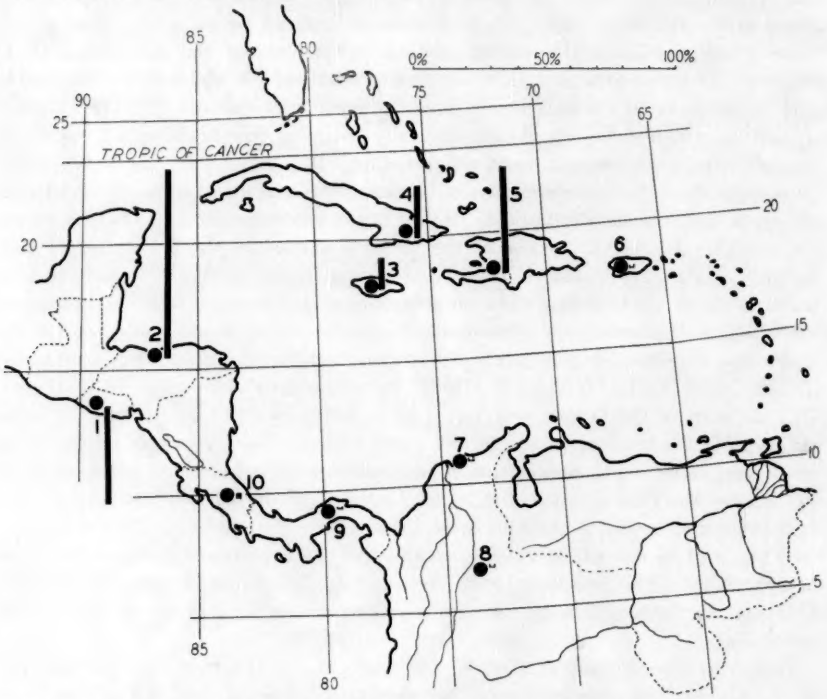


FIG. 1.—Populations of *D. tropicalis*. The heights of the black columns indicate the percentages of the individuals heterozygous for a subbasal inversion in the second chromosome. The scale for the percentages is shown in the upper right. The names of the localities are given in the text.

are smaller than might be desired, it is reasonably clear that only at Lancetilla, Honduras, do the heterozygotes make up significantly more than 50 per cent of the population. In Panama, Columbia, and Brazil[4] this inversion has not been found at all, although some other inversions do occur in the species. Heterozygotes for a submedian inversion in chromosome II L occur in San Salvador and in Honduras;[3] an inversion in chromosome III (shown in Pl. 1 in Burla *et al.*[5]) has been recorded in

the Amazon Valley in Brazil,[4] in two chromosomes from Santa Marta, Columbia, and in a single chromosome from the 1955 sample from San Salvador. Five chromosomes from Santa Marta carried also a short subbasal inversion in chromosome III, and the same inversion was observed in one chromosome from Panama. None of these inversions reaches frequencies anywhere near 50 per cent.

Subandean Populations of Drosophila paulistorum.—In September, 1955, two of the authors (C. P. and Th. D.) collected population samples of *D. paulistorum* on the eastern (Amazonian) slope of the Peruvian Andes, namely, at (1) Urubamba Valley at Huadquiña (elevation, 1,500 meters), (2) Tingo Maria (elevation, 670 meters), and (3) Tarapoto (elevation, 400 meters). In January, 1949, A. G. L. Cavalcanti and one of the authors (Th. D.) collected the same species near Cruzeiro do Sul (4) and at Palmares, near Rio Branco (5), territory of Acre, Brazil. Examination of a single larva in the progeny of each of the wild females collected in nature disclosed that some of them were heterozygous for a short inversion in the third chromosome, described as "K" in a previous report.[4] The proportions of K heterozygotes found are shown in Table 2 and are also represented graphically in Figure 2. The frequency of K heterozygotes of Urumbamba is 79.3 per cent; despite the unfortunately small size of the sample, this is significantly higher than

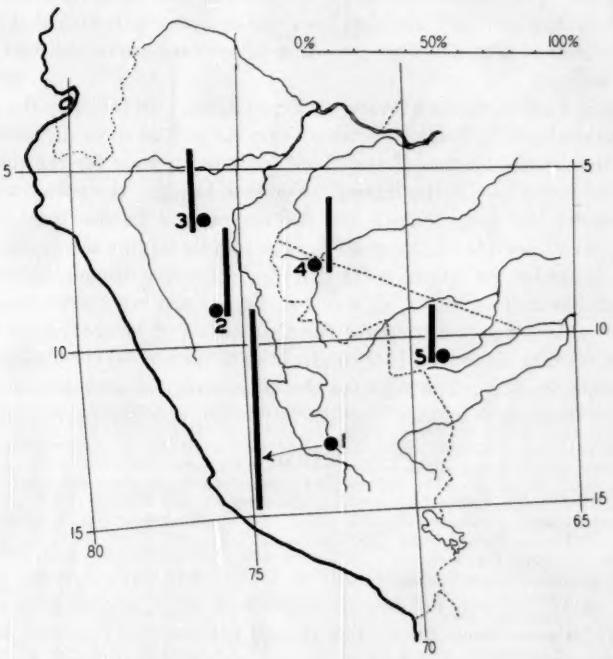


FIG. 2.—Populations of *D. paulistorum*. The black columns indicate the percentages of the inversion K in the third chromosomes in three localities in Peru and in two localities in the territory of Acre in Brazil. The scale for the percentages is shown in the upper right.

50 ± 9.3 per cent of heterozygotes that could occur if the inversion homo- and heterozygotes were equally viable. In the other localities listed above, the frequencies of K are everywhere below 50 per cent. The same inversion has been recorded in scattered individuals in central and southern Brazil.[4] It may be noted that at least 9 different inversions other than K were found in the third chromosomes of the Tingo Maria sample, 8 inversions at Tarapoto, and 3 at Urubamba, but none of these attained frequencies approaching 50 per cent.

TABLE 2

No.	Locality	No. Examined	Per Cent Heterozygotes
1	Urubamba, Peru	29	79.3
2	Tingo Maria, Peru	78	33.3
3	Tarapoto, Peru	26	42.3
4	Cruzeiro do Sul, Brazil	35	28.6
5	Palmares, Brazil	47	21.3

Drosophila willistoni *in Northeastern Brazil.*—Frequencies of inversion heterozygotes exceeding 50 per cent are known only in single populations of *D. tropicalis* and *D. paulistorum* (see above). The territorial extension of the populations in which this remarkable condition is observed remains unknown; the possibility cannot be excluded that this situation may be strictly local and perhaps even impermanent. The populations of *D. willistoni* in northeastern Brazil are, therefore, of more than ordinary interest, since the evidence indicates that populations with frequencies of heterozygotes exceeding 50 per cent are established over a considerable territory.

The map in Figure 3 gives a review of the situation. In this map the percentages in the populations of individuals heterozygous for each of three different inversions are indicated by the heights of the black columns (a scale for the percentages is shown in the lower part of the figure). For each locality, the leftmost of the three columns shows the heterozygosis for the inversion J in the third chromosome (inversion III J; see Da Cunha *et al.*[6]). The middle column shows the frequencies of heterozygotes for the inversion III P, a short inversion discovered in populations of northern Brazil (localities *1*, *2*, and *3* in Fig. 3) and not known elsewhere. Finally, the rightmost column reports the frequencies of heterozygotes for a short, apparently terminal inversion in the right limb of the second chromosome (II R–E; see Da Cunha *et al.*[6]). The data for the critical region (localities *1–8*) are given in Table 3; those for the other localities have been published previously.[6, 7]

TABLE 3

No.	Locality	No. Examined	Per Cent III J	Per Cent III P	Per Cent II R-E
1*A*	Recife, Pernambuco, 1955	84	61.9	27.4	11.9
1*B*	Recife, Pernambuco, 1956	122	60.7	36.1	16.4
1*C*	Olinda, Pernambuco	67	65.7	26.9	13.4
2	João Pessoa, Paraíba	110	63.6	18.2	15.4
3	Natal, R.G. Norte	130	60.8	16.8	20.8
4*A*	Aratuba, Ceará	68	48.5	0	41.2
4*B*	Guaramiranga, Ceará	91	54.9	0	64.8
4*C*	Maranguape, Ceará	37	64.9	0	62.2
5	Rosario, Maranhão	63	47.6	0	7.9
6	São Luiz, Maranhão	100	53.0	0	9.7
7	Castanhal, Pará	62	27.4	0	14.6
8*A*	Belém, Pará, 1949	155	38.7	0	5.2
8*B*	Belém, Pará, 1952	148	37.8	0	8.8

Frequencies of 60 per cent or higher of heterozygotes for the inversion III J have been observed in the populations from Recife northward to Natal (localities *1–3* in Fig. 3). The deviations from the highest theoretically allowed frequency of 50 per cent of heterozygotes are at or above the conventional level of statistical significance in every sample considered by itself and are highly significant if the samples are pooled. The possibility cannot be dismissed outright that the populations were at the time of study not at an equilibrium but in the throes of some

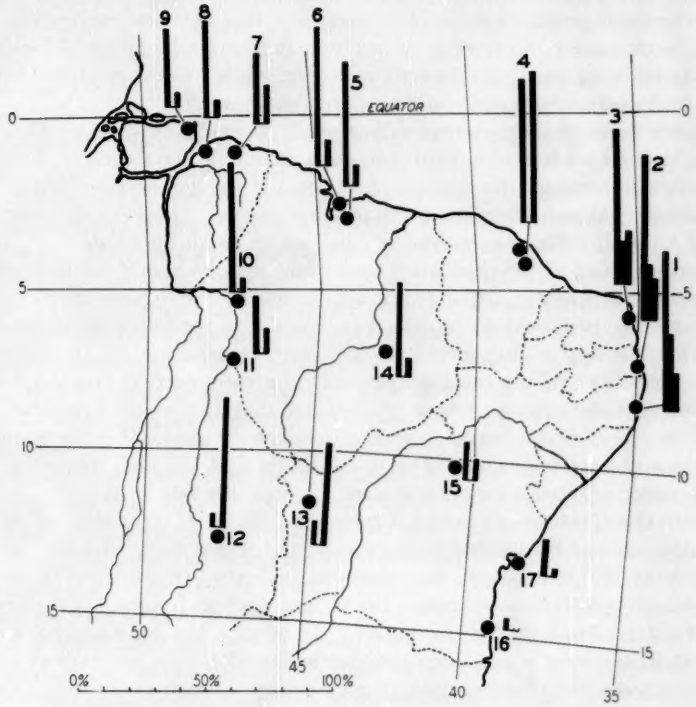


FIG. 3.—Populations of *D. willistoni* in northeastern Brazil. For each locality the height of the leftmost column indicates the frequency of heterozygotes for the inversion J in the third chromosome; the height of the middle column refers to the inversion P in the third chromosome; and the height of the rightmost column pertains to the inversion E in the right limb of the second chromosome. The scale for the percentages is shown in the lower left. The names of the localities are given in the text and in a previous publication (Da Cunha and Dobzhansky, *Evolution*, **8**, 119–134, 1954).

rapid genetic change. The first sample at Recife, and those at Olinda and João Pessoa, were collected by A. G. L. Cavalcanti and one of the authors (Th. D.) in November, 1955. Therefore, Cavalcanti and Dobzhansky repeated the collection at Recife in April, 1956, within a week from making the collection at Natal and in Ceará and Maranhão (localities *3–6*, Fig. 3). The second Recife sample, and that at Natal, had 60 per cent III J heterozygotes, while in Ceará and in Maranhão the frequencies did not deviate significantly from 50 per cent (52.9 per cent in a

total of 359 individuals studied). The frequencies of III J heterozygotes fall rapidly below 50 per cent west of Maranhão (localities *7–9*) and to the south and to the west of Recife (localities *14–17*).

The populations which live from Recife to Natal form a nascent geographic race. The race is characterized by a strongly heterotic inversion III J and by elimination of a considerable proportion of the corresponding homozygotes. It is also characterized by the presence of the inversion III P, which is unknown outside the region where this race lives. Still another characteristic is a moderate frequency of heterozygotes for the inversion II R–E (12–21 per cent); this inversion reaches frequencies possibly significantly in excess of 50 per cent in Ceará (the group of localities *4*, Fig. 3) but falls off rapidly to the west and to the south. Other records of heterotic inversions in populations of *D. willistoni* are also known.[8, 9]

Adaptedness of the Populations with Strongly Heterotic Inversions.—Occurrence of more than 50 per cent of heterozygotes in a panmictic population approximately at equilibrium connotes the elimination of some of the homozygotes owing to differential survival and mortality in the preadult stages. However, the populations affected by such differential mortality need not be handicapped thereby; in fact, the adaptive value of the population as a whole may be high if the heterozygotes possess superior fitness. Although it is not an easy matter to measure the adaptive values of whole populations (see, however, Wallace[10]), the following information is relevant. The four sibling species *D. willistoni*, *D. paulistorum*, *D. equinoxialis*, and *D. topicalis* occur together, sympatrically, in many parts of tropical America. Dobzhansky and Pavlovsky[3] have pointed out that Lancetilla, Honduras, is the only known locality in Central American in which *D. tropicalis* is the commonest of the four sibling species, and it is at Lancetilla that the high frequency of the II L inversion is observed in this species. Among the five localities in Peru and in western Brazil shown in Figure 2, Urubamba (locality *1*) is the only one in which *D. paulistorum* has been found predominant, and it was there that the very high frequency of the inversion K was observed. Finally, *D. willistoni* is decidedly the dominant species in northeastern Brazil, and the high frquency of the inversion III J is observed in a part of this region. To be sure, the dominance of a species in a certain territory is not invariably accompanied by the presence of strongly heterotic inversions. For example, *D. paulistorum* is dominant in Belém (locality *8*, Fig. 3) and in some coastal regions in southern Brazil, and *D. willistoni* is dominant in the state of Baía (localities *15–17*, Fig. 3), without any inversion reaching frequencies above 50 per cent in these regions. However, the evidence shows at least that the elimination of numerous homozygotes does not necessarily incapacitate a population.

Conclusions.—It is not possible at present to evaluate the importance of heterosis in Mendelian populations. The situations reported above are admittedly of a rather special sort. It is chiefly among dipterous insects and in relatively few other organisms that chromosomal inversions are very common in natural populations as carriers of heterotic gene complexes. Even among *Drosophilae*, frequencies of heterozygotes in excess of 50 per cent for any one inversion are uncommon. Nevertheless, some differential elimination of homozygotes and survival of inversion heterozygotes doubtless take place in many natural populations of *Drosophila* in which no one inversion attains the 50 per cent frequency level. Unfortunately,

there is no way at present to estimate how great may be the fraction of zygotes which are sacrificed in *Drosophila* populations to maintain all the heterotic inversions.

Heterosis is, however, widespread also without the association with chromosomal inversions. It is known that in several species of *Drosophila* a great majority of the chromosomes found in natural populations are frankly deleterious when in double dose;[2] they make the homozygotes poorly viable or sterile or both. The adaptive norm of a species is an array of heterotic heterozygotes. How frequently the inviable and sterile homozygotes are produced in nature is unknown, but it is clear that a considerable fraction of the mutational "load" of natural populations of *Drosophila* consists of gene complexes which raise the fitness of heterozygotes but lower that of homozygotes. Such mutant gene complexes constitute a "load" only when and if they become homozygous; with numerous heterotic gene alleles and alternative gene complexes present in the gene pool of a population, the production of homozygotes under panmixia may be low. The incidence of such genes and complexes will be governed by selection to a much greater extent than by mutation rates. How common genes and gene complexes of this sort are in populations of higher animals and man is unknown. It is an open question how much balanced heterosis can be tolerated by populations of a species the fecundity of which is as low as that of man. Many discussions of the genetics of human population ignore the matter completely. Yet Allison's discovery that heterozygosis for the sickling gene in man confers a relative immunity to falciparum malaria[11, 12] is an eloquent testimony of the need for a better understanding of the genetic structure of natural populations.

Summary.—Some of the natural populations of the sibling species *D. tropicalis*, *D. paulistorum*, and *D. willistoni* contain significantly more than 50 per cent of individuals heterozygous for certain inversions. These populations sacrifice a part of the zygotes produced in each generation for the maintenance of the heterotic inversions. Nevertheless, these populatons appear to be highly successful in competition with related species. In *D. willistoni* there is an incipient race occupying a fairly large territory in which about 60 per cent of the individuals are heterozygotes for an inversion in the third chromosome. It is argued that some of the mutational "load" in natural populations of *Drosophila*, and possibly of other organisms, consists of heterotic genes and gene complexes.

Acknowledgments.—The cost of the collecting trips in South America was defrayed in part from the grants made by the Rockefeller Foundation and by the Conselho Nacional de Pesquisas of Brazil to the Department of Biology of the University of São Paulo. We are deeply grateful to Drs. H. L. Carson, W. B. Heed, W. S. Stone, and M. R. Wheeler for sending us the flies of the *willistoni* species group collected by them, to Dr. A. G. L. Cavalcanti, who shared in the collecting work in northeastern Brazil, and to the numerous persons who generously helped our field work in Brazil and in Peru. Mr. and Mrs. B. Spassky and Mrs. O. Pavlovsky have lent their efficient assistance in the maintenance of many laboratory strains and in making the cytological preparations.

* The work reported in this article was supported in part under Contract No. AT-(30-1)-1151, United States Atomic Energy Commission.

[1] H. J. Muller, *Am. J. Human Genet.* **2**, 111–176, 1950.

[2] Th. Dobzhansky and B. Spassky, *Genetics*, **39**, 472–487, 1954.
[3] Th. Dobzhansky and O. Pavlovsky, these PROCEEDINGS, **41**, 289–295, 1955.
[4] Th. Dobzhansky, H. Burla, and A. B. da Cunha, *Am. Naturalist*, **84**, 229–246, 1950.
[5] H. Burla, A. B. da Cunha, A. R. Cordeiro, Th. Dobzhansky, C. Malogolowkin, and C. Pavan, *Evolution*, **3**, 300–314, 1949.
[6] A. B. da Cunha, H. Burla, and Th. Dobzhansky, *Evolution*, **4**, 212–235, 1950.
[7] A. B. da Cunha and Th. Dobzhansky, *Evolution*, **8**, 119–134, 1954.
[8] A. B. da Cunha, *Nature*, **172**, 815, 1953.
[9] A. B. da Cunha, *Evolution*, **10**, 231–234, 1956.
[10] B. Wallace, *J. Genet.*, **54**, 280–293, 1956.
[11] A. C. Allison, *Ann. Human Genet.*, **19**, 39–57, 1954.
[12] A. C. Allison, *Cold Spring Harbor Symposia Quant. Biol.*, **20**, 239–255, 1955.

REGULARITY THEOREMS FOR SOLUTIONS OF PARTIAL DIFFERENTIAL EQUATIONS WITH VARIABLE COEFFICIENTS

BY FELIX E. BROWDER

DEPARTMENT OF MATHEMATICS, YALE UNIVERSITY

Communicated by Einar Hille, December 10, 1956

Let G be a domain, A a partial differential operat or with coefficients defined on G. During the past several years, regularity theorems have been proved for weak solutions of $Au = f$ when A is elliptic or parabolic.[1] For operators with constant coefficients, L. Hörmander[2] has given an algebraic characterization of the complete operator of local type, the class for which the Weyl lemma is valid, i.e., that every weak solution of $Au = 0$ is infinitely differentiable in G. It is the purpose of this note to present a proof of a regularity theorem for weak solutions for a general class of operators with variable coefficients which for the special case of constant coefficients coincides with the complete operators of local type, while the general case includes the elliptic and parabolic operators with variable coefficients.[3] For formally self-adjoint operators of this class, we obtain as a consequence an eigenfunction expansion theorem with differentiable eigenfunctions.[4] For simplicity of presentation our regularity theorem is given for operators with infinitely differentiable coefficients, although much weaker hypotheses would suffice.

I. Let G be an open set in E^n with co-ordinates $(x_1, \ldots, x_n)$, $(,)$ the inner product in $L^2(G)$, $C_c^\infty(G)$ the infinitely differentiable functions with compact support in G. R^n will be the dual space of E^n with its elements denoted by $\xi = (\xi_1, \ldots, \xi_n)$, $[x, \xi] = \sum_j x_j \xi_j$ for $x \, \xi \, E^n$, $\xi \in R^n$, $|\xi|^2 = \sum \xi_j^2$. In E^n we define $D_j = (i)^{-1}(\partial/\partial x_j)$, while for $h \in E^n$ with none of its components zero, $V_h^j u(x) = (h_j)^{-1}\{u(x + h_j) - u(x)\}$, $M_h^j u(x) = {}^1/_2\{u(x + h_j) + u(x)\}$. In R^n, $D_\xi^j = \partial/\partial \xi_j$. For the multi-index $\alpha = (\alpha_1, \ldots, \alpha_k)$, $1 \leq \alpha_j \leq n$, $D^\alpha = D_{\alpha_1} \ldots D_{\alpha_k}$, $|\alpha| = k$, $V_h^\alpha = V_h^{\alpha_1} \ldots V_h^{\alpha_k}$, $M_h^\alpha = M_h^{\alpha_1} \ldots M_h^{\alpha_k}$, $D_\xi^\alpha = D_\xi^{\alpha_1} \ldots D_\xi^{\alpha_k}$, $\xi^\alpha = \xi_{\alpha_1} \ldots \xi_{\alpha_k}$.

Let A be a partial differential operator of order m, $A = \sum a_\alpha(x) D^\alpha$, where, for each α, a_α belongs to $C^{|\alpha|}(G)$. Its formal adjoint is $A^+ u = \sum D^\alpha(a_\alpha(x) u)$, while the characteristic polynomial of A is $A(x, \xi) = \sum_\alpha a_\alpha(x) \xi^\alpha$. Let $A^\beta(x, \xi) = D_\xi^\beta A(x, \xi)$, while $A_{x_0} = \sum_\alpha a_\alpha(x_0) D^\alpha$ if $x_0 \in G$. If P and Q are differential operators with

constant coefficients, Q is weaker than P ($Q < P$) if there exists $K > 0$ such that $|Q(\xi)| \leq K\sum|P^\beta(\xi)|$, $\xi \in R^n$, and strictly weaker than P ($Q \ll P$) if $|Q(\xi)\xi| \leq K\sum|P^\beta(\xi)|$, $\xi \in R^n$.

II. If $\psi \in L^2(G)$, then $u \in L^2(G)$ is said to be a weak solution of $Au = \psi$ in G if $(u, A^+\phi) = (\psi, \phi)$ for $\phi \xi C_c^\infty(G)$. In the case of constant coefficients Hörmander[2] has shown that all weak solutions of the equation $Au = 0$ are in $C^\infty(G)$ after correction on a null set if and only if the following condition is fulfilled:

(π) For $|\beta| > 0$, $A^\beta(\xi) = o(|A(\xi)|$ as $|\xi| \to \infty$ in R^n.

THEOREM. *Let A be a differential operator on G with its coefficients in $C^\infty(G)$. Suppose that for each x_0 in G, $A_0 = A_{x_0}$ satisfies (π) while there is a neighborhood N of x_0 in G such that for $x \in N$, $A = A_0 + \sum c_\beta(x)Q_\beta$, the sum being taken over a finite set of Q_β such that $Q_\beta < A_0$, $Q_\beta^\alpha \ll A_0$ for $|\alpha| > 0$. If u is a weak solution of $Au = \psi$ in G with $\psi \in C^\infty(G)$, then, after correction on a null set, u will lie in $C^\infty(G)$.*

Remark 1: Simple examples of operators satisfying the conditions of the theorem are (i) elliptic operators; (ii) operators of the form $B(x, t)(\partial/\partial t) + A(x, t)$ in E^{n+1} with co-ordinates $(x_1, \ldots, x_n, t)$, where, for each fixed t, $B(x, t)$ and $A(x, t)$ are elliptic operators in the x-variables.

Proof of the Theorem: Following the lines of Hörmander's argument using Fourier transforms of distributions,[5] one constructs a fundamental solution E for A_0^+, i.e., a distribution such that $A_0^+(E*\phi) = \phi$ for $\phi \in C_c^\infty(E^n)$, for which in addition we have the following: (*a*) If $u \in L^2(E^n)$ with compact support, G_1 a compact in E^n, $|Q(\xi)| \leq C'\sum|A_\alpha(\xi)|$ for $\xi \in R^n$, then there exists $C_2 > 0$ depending only on C', G_1, and the support of u such that $\|Q(D)(E * u)\|_{G_1} \leq C_2\|u\|$. (*b*) If $Q \ll P$, $1 \leq j \leq n$, then $\|D_j(E * u)\|_{G_1} \leq C_2\|u\|$. (*c*) E belongs to C^∞ in the complement of 0.

The hypothesis implies that, in N, $A^+ = A_0^+ + \sum c_\beta'(x)Q_\beta$, where $c_\beta'(x) \to 0$ as $x \to x_0$. There exists $\lambda > 0$ such that if $N\lambda = \{x|x - x_0| < \lambda\}$, $\sum\|c_\beta'Q_\beta(E * \phi)\|N_\lambda \leq {}^1/_2\|\phi\|$ for $\phi \in C_c^\infty(N_\lambda)$. Let $p \in C_c^\infty(E^n)$ with its support in N_λ, $p = 1$ on $N_{\lambda/2}$. Suppose first that v satisfies the equation

$$(v, A^+\phi) = \sum_\beta(\psi_\beta, Q_\beta\phi),\ \phi \in C_c^\infty(N_\lambda);\quad v, \psi_\beta \in L^2(N_\beta). \tag{1}$$

Then

$$\sum_\beta(\psi_\beta, Q_\beta p^{2m}(E * \phi)) = (v, p^{2m}A^+(E * \phi)) + \sum_{Q_\beta << A_0}(v, p^{s(\beta)}c_\beta''Q_\beta(E * \phi))$$
$$= (p^{2m}v, \phi)\sum_{Q\beta \ll A_0}(p^{2m}, c_\beta'Q_\beta(E * \phi)) + \sum_{Q\beta << A_0}(v, p^{(s)\beta}c_\beta''Q_\beta(E * \phi)). \tag{2}$$

We note that by (*b*) above, $\sum_{Q\beta << A_0}(v, p^{s(\beta)}c_\beta''Q_\beta(E * \phi)) = (R * (cv))$, where $\|D_j(R * cv)\|_{N\lambda} \leq C_3\|v\|_{N\lambda}$, $1 \leq j \leq n$. Since $v \in L^2(N_\lambda)$, while $C_c^\infty(N_\lambda)$ is dense in $L^2(N_\lambda)$, from equation (2) we obtain the inequality

$$\|v - R * (cv)\|_{\lambda/2} \leq C_4\sum_\beta\|\psi_\beta\|N_\lambda. \tag{3}$$

Suppose now that u is a weak solution of $Au = \psi$ in $N_{2\lambda}$. If $|\alpha| \cdot |h| < \lambda$, $\phi \in C_c^\infty(N_\lambda)$, then

$$(V_h^\alpha u, A^+\phi) = (u, A^+(V_{-h}^\alpha\phi)) + \sum_{\substack{\beta;\ \alpha=(\xi,\eta)\\ |\xi|>0}} m_{\eta\xi}(u, (M_{-h}^\eta V_{-h}^\xi c_\beta')(M_{-h}^\xi V_{-h}^\eta Q_\beta\phi))$$
$$= (V_h^\alpha\psi, \phi) + \sum_{|\eta|<|\alpha|} m_\eta'(V_h^\eta u, (V_{-h}^\xi c_\beta')(M_{-h}^\xi Q_\beta\phi)). \tag{4}$$

If we have shown that for $|\eta| < |\alpha|$, $V_h^{\eta}u$ for sufficiently small h have their $L(N_2-|\alpha|+1_\lambda)$-norms uniformly bounded in h and since the same is true for $V_h^{\alpha}\psi$ and $V_h^{\xi}c_\beta'$, we may apply relation (3) to $v = V_h^{\alpha}u$ and conclude that $\|V_h^{\alpha}u\|_{(N-|\alpha|_\lambda)}$ is uniformly bounded in h for all sufficiently small h. But as $h \to 0$, $V_h^{\alpha}u$ converges in the distribution sense to $(\partial/\partial x)^{\alpha}u$. If we consider the sequence $V_{2-\eta_h}u$ in $L^2(N_2-|\alpha|_\lambda)$, then, by the uniform boundedness of the norms, we can find a subsequence converging weakly to an element of $L^2(N_2-|\alpha|_\lambda)$ which must equal $(\partial/\partial x)^{\alpha}u$ in that neighborhood. Since all the distribution derivatives of u are locally in L^2, our conclusion follows from a well-known theorem of Sobolev.[6]

Remark 2: By a refinement of the argument, we can remove the restriction that $u \in L^2(G)$ and prove regularity for any distribution solution. Similarly, bounds may be obtained for the L^2-norms of u and its derivatives on a compact G_1 in terms of L^2-norms of ψ and its derivatives and any negative Dirichlet norm of u on any G_2 containing $\bar{G}_1$ in its interior.

[1] For a survey of recent results in the elliptic case cf. F. E. Browder, *Communs. Pure and Appl. Math.*, **9**, 351–361, 1956; L. Nirenberg, *Communs. Pure and Appl. Math.*, **9**, 509–529, 1956. For the parabolic case see F. E. Browder, these PROCEEDINGS, **42**, 914, 1956.

[2] L. Hörmander, *Acta math.*, **94**, 161–248, 1955.

[3] The first result of this type was stated by Hörmander.

[4] F. E. Browder, these PROCEEDINGS, **42**, 769–771, 1956.

[5] Hörmander, *op. cit.*, pp. 222–229.

[6] Cf. L. Schwartz, *Théorie des distributions* (Paris, 1951), **2**, 47, Theorem XIX.

TWO RECURSIVELY ENUMERABLE SETS OF INCOMPARABLE DEGREES OF UNSOLVABILITY (SOLUTION OF POST'S PROBLEM, 1944)

BY RICHARD M. FRIEDBERG

HARVARD UNIVERSITY

Communicated December 11, 1956

Post[1] has questioned the existence of a recursively enumerable set of non-negative integers which is neither recursive nor of the highest degree of unsolvability possible to recursively enumerable sets. This question is now answered by the construction of two recursively enumerable sets of which we prove that neither is recursive in the other and hence that they both satisfy the criteria of Post's question. In the theorem we shall deal not with the sets directly but with their representing functions (functions taking the values 0 and 1, respectively, for members and nonmembers of the sets).[2]

THEOREM I. *There exist two functions f_1 and f_2, both of which represent recursively enumerable sets and neither of which is recursive in the other.*

We shall define f_1 and f_2 by successive approximation through a pair of sequences of functions $f_1^0, f_1^1, f_1^2, \ldots$ and $f_2^0, f_2^1, f_2^2, \ldots$. For each pair of numbers a, e we shall define a number $x_1^a(e)$ for which we intend to set $f_1^{a'}(x_1^a(e)) = 0$ if at some later stage a' $(\geq a)$ of the construction we encounter a y for which

$$T_1^{f_2^{a'-1}}(e, x_1^a(e), y) \;\&\; U(y) = 1, \qquad (1)^3$$

and otherwise to leave $f_1^{a'}(x_1^a(e))$ unchanged as a' increases. Once relation (1) is established, we shall act so as to insure as nearly as possible that it will remain true for all higher a', and hence also with f_2 in place of $f_2^{a'-1}$. Similarly for $x_2^a(e)$, with f_1^a and f_2^a interchanged.

The functions $f_1^a(x)$, $f_2^a(x)$, $x_1^a(e)$, and $x_2^a(e)$ shall be recursive as two-argument functions. We define them as follows.

Case 0: $a = 0$. Let

$$f_1^0(x) = f_2^0(x) = 1, \qquad \text{all } x;$$

$$x_1^0(e) = x_2^0(e) = 2^e, \qquad \text{all } e.$$

Case 1: $a = 2b + 1$. Let e_a be the number of prime divisors of b (so that for any fixed e we have $e_a = e$ for infinitely many a). Two subcases are possible.

Subcase 1.1: $f_1^{a-1}(x_1^{a-1}(e_a)) = 1$ and $(Ey < a)[T_1^{f_2^{a-1}}(e_a, x_1^{a-1}(e_a), y)\ \&\ U(y) = 1]$. Then let

$$f_1^a(x_1^{a-1}(e_a)) = 0;$$

$$x_2^a(e) = 2^e \cdot (2a + 1), \qquad \text{all } e \geqslant e_a;$$

and otherwise let

$$f_1^a = f_1^{a-1}, \qquad f_2^a = f_2^{a-1}, \qquad x_1^a = x_1^{a-1}, \qquad x_2^a = x_2^{a-1}.$$

Subcase 1.2: Otherwise. Then let

$$f_1^a = f_1^{a-1}, \qquad f_2^a = f_2^{a-1}, \qquad x_1^a = x_1^{a-1}, \qquad x_2^a = x_2^{a-1}.$$

Case 2: $a = 2b + 2$. Treat as Case 1 with subscripts 1 and 2 interchanged and with "$e \geqslant e_a$" in line 4 of Subcase 1.1 replaced by "$e > e_a$" in Subcase 1.2.

This completes the definition of the auxiliary functions. Now let $f_1(x) = 0$ or 1 according as $(Ea)(f_1^a(x) = 0)$ or not, and similarly for f_2. Clearly, f_1 and f_2 represent recursively enumerable sets.

Note that the only information about a function f relevant to the statement $T_1^f(e, x, y)$ is information about its values on arguments $u < y$ (because all the relevant arguments occur in a formal expression with Gödel number y). Therefore, the changes made in x_2^a under Subcase 1.1 prevent the falsification of $T_1^{f_2^{a'}}(e_a, x_1^a(e_a), y)$ for any $a' \geq a$ except through an occurrence of Subcase 2.1 with $e_{a'} < e_a$.

The success of the construction depends upon two lemmas.

LEMMA I. *For any given e, $x_1^a(e)$ changes only a finite number of times as a increases through the natural numbers.*

Because there are only finitely many $e < \bar{e}$, the lemma can fail for $e = \bar{e}$ only if, for some fixed $\bar{e}' < \bar{e}$, Subcase 2.1 occurs an infinite number of times with $e_a = \bar{e}'$. Since each such occurrence changes $f_2^a(x_2^a(\bar{e}'))$ from 1 to 0, this in turn requires infinitely many changes of $x_2^a(\bar{e}')$. But this, by similar reasoning, requires infinitely many changes of $x_1^a(e)$ for some fixed $e \leqslant \bar{e}' < \bar{e}$. Therefore, $\bar{e}$ is not the smallest number for which the lemma fails. Thus the lemma is proved by induction.

LEMMA II. *Let $z_1(e)$ be the last (unchanged) value assumed by $x_1^a(e)$ as a increases. Then $(Ey)[T_1^{f_2}(e, z_1(e), y)\ \&\ U(y) = 1] \equiv f_1(z_1(e)) = 0$.*

For if $T_1^{f_2}(e, z_1(e), y)$ & $U(y) = 1$ for some y, then (since, for sufficiently high a, $f_2^a(u) = f_2(u)$, all $u < y$) $f_1^a(z_1(e))$ will eventually be made equal to zero through an occurrence of Subcase 1.1.

Conversely, if $f_1(z_1(e)) = 0$, Subcase 1.1 must have arisen for some a for which $x_1^a(e) = z_1(e)$ and $(Ey)[T_1^{f_2^{a-1}}(e, z_1(e), y)$ & $U(y) = 1]$. No occurrence of Subcase 2.1 with $e_a < e$ can subsequently falsify this latter statement, for such an occurrence would induce a change in $x_1^a(e)$, contrary to the definition of $z_1(e)$. Therefore, the statement remains true with f_2 in place of f_2^{a-1}.

Lemmas I and II hold for any e and as well with f_1 and f_2 interchanged. Therefore, neither f_1 nor f_2 is recursive in the other.[4]

THEOREM II. *Given a set A, there exist two sets not recursive in one another, both enumerable by a procedure recursive in A and both of degree higher than that of A.*

Proof: Change Case 0 in Theorem I to make $f_1^0(2^e) = f_2^0(2^e) = 0$ instead of 1 whenever e belongs to A, and to make $x_1^0(e) = x_2^0(e) = 3 \cdot 2^e$ for all e. Then f_1 and f_2 represent the desired sets.

[1] Emil L. Post, "Recursively Enumerable Sets of Positive Integers and Their Decision Problems," *Bull. Am. Math. Soc.*, **50**, 284–316, 1944.

[2] S. C. Kleene and Emil L. Post ("The Upper Semi-lattice of Degrees of Recursive Unsolvability," *Ann. Math.*, **59**, 379–407, 1954) produce two functions neither of which is recursive in the other. The present paper adapts their method to the restriction that both functions represent recursively enumerable sets. The gist of this adaptation was presented by title at the February 25, 1956, meeting of the American Mathematical Society and has been abstracted (R. Friedberg, *Bull. Am. Math. Soc.*, **62**, 260, 1956, Abstr. 362).

[3] U is a recursive function, and T_1^f is a predicate which is recursive if f is, such that $T_1^f(e, x, y)$ means that e is the Gödel number of a formal procedure for calculating one function, given another; that y is the Gödel number of a formal application of this procedure with f as the given function; and that this application yields the value $U(y)$ for the calculated function on the argument x. (See S. C. Kleene, *Introduction to Metamathematics* [New York: D. Van Nostrand Co., 1952], pp. 276–278 and 288–291.) Hence f_1 cannot be recursive in f_2 if for each e there is an x such that

$$f_1(x) \neq 1 \equiv (Ey)[T_1^{f_2}(e, x, y) \text{ \& } U(y) = 1].$$

[4] The nonconstructiveness of Lemma I, which demonstrates that z_1 is defined for all arguments without telling us how to calculate it, is a necessary feature of the construction. For if z_1 were recursive, f_1 would represent a creative set and would therefore, by a theorem of J. R. Myhill ("Creative Sets," *Z. math. Logik u. Grundlagen Math.*, **1**, 97–108, 1955), be of the highest degree possible to recursively enumerable sets.

A full exposition of this theorem is to appear in two forthcoming texts: H. Rogers, *Theory of Recursive Functions and Effective Computability* (mimeo), MIT Math. Dept., Cambridge, 1957; and J. C. E. Dekker and J. R. Myhill, *Recursion Theory*.

A THEOREM ON STABILITY OF COMPLEX STRUCTURES

By Alfred Frölicher and Albert Nijenhuis

INSTITUTE FOR ADVANCED STUDY, PRINCETON, NEW JERSEY, AND UNIVERSITY OF WASHINGTON, SEATTLE, WASHINGTON

Communicated by O. Veblen, December 17, 1956

Let X be a compact manifold with a given differentiable structure. Any complex structure J is determined by its almost-complex structure tensor, also denoted by J. Two complex structures J and J' are called equivalent if there is a differentiable homeomorphism Ψ: $X \to X$ such that $J' = \Psi^* J$, where Ψ^* denotes the induced mapping of the tensor fields over X. Let, further, $\Theta_0(J)$ denote the sheaf of germs of holomorphic vector fields over X with respect to a complex structure J.

THEOREM. *If J_t, $0 \leq t \leq 1$, is a C^1 family of complex structures on a compact manifold X, and if $H^1(X, \Theta_0(J_0)) = 0$, then there exists a positive number ϵ such that for all t satisfying $0 \leq t \leq \epsilon$ the complex structures J_0 and J_t are equivalent.*

The proof shows that if $\dim H^1(X, \Theta_0(J_t)) = 0$ for $0 \leq t \leq 1$, then J_0 and J_t are equivalent for *all* t, $0 \leq t \leq 1$.

A forthcoming paper of Bott will show that $H^1(X, \Theta_0(J)) = 0$ for every compact homogeneous Kähler manifold with finite fundamental group. This gives

COROLLARY. *If J_t, $0 \leq t \leq 1$, is a C^1 family of complex structures on a compact manifold X with finite fundamental group, and if J_0 is a homogeneous Kähler structure, then there exists a positive ϵ such that J_0 and J_t are equivalent for all $0 \leq t \leq \epsilon$.*

The corollary covers the case of deformation of the complex structure of complex projective space. This special result was simultaneously and independently obtained by Kodaira and Spencer. Their proof, to appear in a continuation of another paper,[1] uses quite different methods.

The proof of the theorem is preceded by three lemmas.

LEMMA 1. *If $H^1(X, \Theta_0(J_0)) = 0$, then there is an $\epsilon > 0$ and a continuous family of vector fields v_t, $0 \leq t \leq \epsilon$, such that $dJ_t/dt = [J_t, v_t]$.*

Proof: (The subscript t is dropped where no confusion will arise.) The groups $H^q(X, \Theta_p(J))$, as defined earlier,[2] are the cohomology groups of the coboundary operator $\mathfrak{D}$: $L \to [J, L]$ developed in another paper,[3] where L is a vector form of type $(p, q)'$ in the sense of Paper C. I.;[2] i.e., L is a tensor with components $L^{\alpha}_{\beta_1 \cdots \beta_p \bar{\gamma}_1 \cdots \bar{\gamma}_q}$ with respect to complex co-ordinates $(z^1, \ldots, z^n, \bar{z}^1, \ldots, \bar{z}^n)$, skew-symmetric in the β's and the γ's. Let $P = {}^1/_2(I - iJ)$; then $A' = P(dJ/dt)$ $(= P \mathbin{\overline{\wedge}} dJ/dt$ in the notation of Paper C. I.) is a vector form of type $(0, 1)'$, as follows from $JJ = -I$ by differentiation, using Proposition 6 of Papers I and II. Differentiating $[J, J] = 0$, we obtain $[J, dJ/dt] = 0$, and the part of type $(0, 2)'$ of the left-hand side of this equation gives $\mathfrak{D}A' = [J, A'] = [J, P(dJ/dt)] = 0$. Hence A' represents a cohomology class of $H^1(X, \Theta_0(J))$. If this class is the zero class, then there exists a complex vector field v' of type $(0, 0)'$ such that $A' = [J, v']$.

We can choose a differentiable family of Riemannian metrics $g = g_t$ such that g_t is hermitian with respect to $J = J_t$. Let $\bar{\vartheta}$ be the adjoint operator of $\mathfrak{D}$ with respect to g; Δ the Laplacian $\Delta = \mathfrak{D}\bar{\vartheta} + \bar{\vartheta}\mathfrak{D}$; and G Green's operator. The decomposition theorem of Kodaira[4] then gives $A' = \mathfrak{D}\bar{\vartheta}GA' + \bar{\vartheta}\mathfrak{D}GA' + HA'$,

where HA' is the harmonic part of A'. A theorem of Kodaira and Spencer[1] implies that $h(t) = \dim H^1(X, \Theta_0(J_t))$ is upper semicontinuous in t. Since $h(0) = 0$, there is a positive ϵ such that $h(t) = 0$ for $0 \leq t \leq \epsilon$. Then also the dimension of the space of harmonic vector forms of type $(0, 1)'$ vanishes; in particular, $H_t A_t' = 0$ for $0 \leq t \leq \epsilon$. Another theorem of Kodaira and Spencer (to appear in a continuation of the paper[1] quoted above) states that G_t is differentiable in t whenever $h(t)$ is constant. Hence G_t is differentiable in t for $0 \leq t \leq \epsilon$. Because $\mathfrak{D}A' = 0$ and $\mathfrak{D}G = G\mathfrak{D}$, it follows that $A' = \mathfrak{D}\bar{\vartheta}GA'$; hence $A' = \mathfrak{D}v' = [J, v']$, where $v' = \bar{\vartheta}GA'$ is continuous in t. Put $v = v' + \bar{v}'$, and note that $dJ/dt = A' + \bar{A}'$, $\bar{A}' = [J, \bar{v}']$, hence $dJ/dt = [J, v]$, where $v = v_t$ is real and continuous in t. This proves the lemma.

Remark: The expression $[v, J] = -[J, v]$ is the Lie derivative of J with respect to v (cf. an earlier paper[3]). For the Lie derivative of any tensor T the notation $[v, T]$ will be used.

LEMMA 2. *If v_t, $0 \leq t \leq 1$, is a continuous family of vector fields over X, then there exists a differentiable family of homeomorphisms Ψ_t: $X \to X$ such that, for every tensor field T, $(d/dt)(\Psi_t^* T) = -[v_t, \Psi_t^* T]$, $0 \leq t \leq 1$.*

Proof: Let u_t be a continuous family of vector fields over X, and let Φ_t: $X \to X$ be the family of homeomorphisms determined by the local co-ordinate equations $(d/dt)\Phi_t^i = u_t^i(\Phi_t^1, \ldots, \Phi_t^n)$, $i = 1, \ldots, n$, with initial values $\Phi_0^i = x^i$; i.e., $\Phi_0(x) = x$ for all $x \in X$. Φ_t is said to be the exponential of u_t, and u_t the tangent field of Φ_t. One can easily prove $(d/dt)(\Phi_t^{*-1} T) = \Phi_t^{*-1}[u_t, T]$ for every tensor field T, as a direct consequence of the definition of Lie derivatives. Since the Lie operations commute with mappings, we have $\Phi_t^{*-1}[u_t, T] = [\Phi_t^{*-1} u_t, \Phi_t^{*-1} T]$. Let Ψ_t be the exponential of the given v_t, and $\Phi_t = \Psi_t^{-1}$, while u_t is the tangent field of Φ_t. If x^i are local co-ordinates of x, and y^j local co-ordinates of $y = \Phi_t(x)$, then $y^j = \Phi^j(t, x^1, \ldots, x^n)$ and $x^i = \Psi^i(t, y^1, \ldots, y^n)$, $i, j = 1, \ldots, n$. Therefore, $dy^j = (\partial\Phi^j/\partial t)dt + \sum_i (\partial\Phi^j/\partial x^i)dx^i$ and $dx^i = (\partial\Psi^i/\partial t)dt + \sum_j (\partial\Psi^i/\partial y^j)dy^j$, from which follows $\partial\Psi^i/\partial t = -\sum_j (\|\partial\Phi/\partial x\|^{-1})_j^i(\partial\Phi^j/\partial t)$, i.e., $v_t = -\Phi_t^{*-1} u_t$. Hence we have $(d/dt)(\Psi_t^* T) = (d/dt)(\Phi_t^{*-1} T) = [\Phi_t^{*-1} u_t, \Phi_t^{*-1} T] = -[v_t, \Psi_t^* T]$. *Q.E.D.*

LEMMA 3. *Let S_t and T_t be two differentiable families of tensor fields satisfying $dY/dt = -[v_t, Y]$, v_t being a continuous family of vector fields. If $S_0 = T_0$, then $S_t = T_t$, $0 \leq t \leq 1$.*

Proof: Introduce a positive definite Riemann metric on X, and let $g(Y, Y)$ be the square norm of the tensor Y. Put $D_t Y = dY/dt + [v_t, Y]$, and take $Y = S_t - T_t$. Then $D_t(g(Y, Y)) = 2g(DY, Y) + D_t g(Y, Y) = [v_t, g](Y, Y)$. A compactness argument shows that there is an $M > 0$ such that $|[v_t, g](Y, Y)| < Mg(Y, Y)$ for any tensor Y of the particular type. Hence, putting $g(Y, Y) = y_t$, one has $|D_t y_t| < M|y_t|$; $y_0 = 0$; and D_t denotes differentiation in $X \times I$ along the trajectories of the vector field $(v_t, 1)$. A standard argument shows that $y_t = 0$ along every trajectory, hence $y_t = 0$ on $X \times I$; and consequently $S_t = T_t$. *Q.E.D.*

Proof of the theorem: Both J_t and $\Psi_t^* J_0$, $0 \leq t \leq \epsilon$, satisfy $dY/dt = [-v_t, Y]$, $Y_0 = J_0$ (Lemmas 1 and 2); hence, by Lemma 3, $J_t = \Psi_t^* J_0$, which proves the theorem.

[1] K. Kodaira and D. C. Spencer, "On the Variation of Almost-complex Structures," in *Algebraic Geometry and Topology* (Princeton, N.J., 1957).

[2] A. Frölicher and A. Nijenhuis, "Some New Cohomology Invariants for Complex Manifolds. I, II." *Indagationes math.*, **18**, No. 5, 1956 (here referred to as Paper C. I.).

[3] A Frölicher and A. Nijenhuis, "Theory of Vector-valued Differential Forms. I," *ibid.* No. 3, pp. 338–359, 1956.

[4] K. Kodaira, "On a Differential Geometric Method in Theory of Analytic Stacks," these PROCEEDINGS, **39**, 1268–1273, 1953.

ON THE SPECTRAL SEQUENCE OF A FIBER SPACE. II

BY WITOLD HUREWICZ AND EDWARD FADELL*

MASSACHUSETTS INSTITUTE OF TECHNOLOGY AND UNIVERSITY OF WISCONSIN

Communicated by Richard Brauer, December 18, 1956

1. INTRODUCTION

The objective of this paper is to outline a proof of the following result, first conjectured by Hurewicz, which generalizes a well-known theorem of Leray-Serre[1] and also extends a previous result of the authors.[2] The details will appear elsewhere.

THEOREM. *If (X, B, p) is a fiber space in the sense of Hurewicz[3] with fiber F, and if B is $r-1$-connected, $r > 1$, then, in the associated spectral sequence $\{E_j, d_j\}$ based on singular chains with coefficient group G, we have the following:*

1. $E_i \backsimeq H(B, H(F, G)), \quad 2 \leq i \leq r; \quad d_i = 0, \quad 2 \leq i \leq r-1.$

2. *Identifying $H(B, H(F, G))$ with E_r, the differential operator d_r: $H(B, H(F, G)) \to H(B, H(F, G))$ is given by the cap product*

$$d_r(h) = \gamma \cap h, \qquad h \in H(B, H(F, G)),$$

where γ is the characteristic cohomology class of B and $H(F, G)$ and $\pi_r(B)$ are suitably paired.

It should be remarked that the authors first proved, independently, that under the hypotheses of the above theorem d_r depends only on the pairing of $H(F, G)$ and $\pi_r(B)$—i.e., the "action" of a higher homotopy group on the homology of F. The simple description of d_r in terms of cap products was suggested by Norman Steenrod.

2. PRELIMINARIES

2.1. *Fiber Spaces.*—We employ the concept of fiber space as given by Hurewicz.[4]

2.2 *The Spectral Sequence Based on Singular Prisms.*—Let X denote a topological space and $\Delta^n = |v_0, \ldots, v_n|$ a fixed n-simplex, for $n = 0, 1, \ldots$. A (p, q) singular prism α is a map $\alpha: \Delta^p \times \Delta^q \to X$. The free Abelian group generated by all such singular prisms will be denoted by $\hat{C}(X)$. If G is an arbitrary coefficient group, we set $\hat{C}(X, G) = \hat{C}(X) \otimes G$. Introducing the usual boundary operator ∂ in $\hat{C}(X, G)$, we obtain a chain complex. Furthermore, we adopt the usual convention that when the symbol G is omitted, integral coefficients are assumed.

Now let (X, B, p) denote a fiber space, and let $\hat{C}(X)$ denote the singular chains of X based on prisms.[5] Let $A(X)$ denote the subcomplex of $\hat{C}(X)$ generated by (m, n) prisms α such that $p\alpha$: $\Delta^m \times \Delta^n \to B$ depends only upon the second co-ordinate, i.e., $p\alpha(x, y) = p\alpha(x', y)$. $A(X)$ is seen to be chain equivalent to $\hat{C}(X)$. We filter $A(X)$ as follows. $A^p(X) = A^p$ is generated by (m, n) singular prisms in $A(X)$ such that $n \leq p$. The spectral sequence $\{E_j, d_j\}$ associated with (X, B, p) can then be defined as follows. $E_j^{p,q}, j \geq 1$, is the image of the homomorphism

$$i_*: H_{p+q}(A^p, A^{p-j}) \to H_{p+q}(A^{p+j-1}, A^{p-1}),$$

where i_* is induced by the injection $A^p \subset A^{p+j-1}$. Then $E_j^p = \sum_q E_j^{p,q}$ and $E_j = \sum_p E_j^p$. The differential operator d_j: $E_j^{p,q} \to E_j^{p-j,q+j-1}$ is given by restricting ∂: $H_{p+q}(A^{p+j-1}, A^{p-1}) \to H_{p+q-1}(A^{p-1}, A^{p-j-1})$ to $E_j^{p,q}$. In what follows, we may use the notation $E_j(X)$ for E_j to avoid confusion when more than one fiber space is involved. Also, if we use a coefficient group other than Z (= integers), we may write $E_j(X, G)$.

Let $F = p^{-1}(b)$, $b \in B$, denote a fiber of X. Then $C(B)$, $C(F)$ will denote the singular chains of B and F, respectively, based on simplexes. If B is r-connected, $r \geq 0$, then we may assume that $C(B)$ is generated by singular simplexes whose r-skeleton lies at b, and $A(X)$ is generated by singular prisms whose r-skeleton lies in F. We will throughout assume that B is 0-connected and hence that singular elements employed have vertices at b or in F, respectively. If s is a singular (m, n) prism in A^p, we associate with s two singular simplexes Fs and Bs as follows. Fs: $\Delta^m \to F$ is given by $Fs(x) = f(x, v_0)$, $x \in \Delta^m$, where v_0 is the leading vertex of Δ^n. Bs: $\Delta^n \to B$ is given by $Bs(y) = ps(x, y)$, $y \in \Delta^n$, and x arbitrary in F if $n = p$; otherwise, $Bs = 0$. Set $\theta(s) = Fs \otimes Bs$. Thus we obtain a chain map

$$\theta: A^p/A^{p-1} \to C(F) \otimes C_p(B),$$

where the boundary operator in $C(F) \otimes C_p(B)$ is given by $\partial(c \otimes c') = \partial c \otimes c'$. θ, of course, depends upon p.

2.3. *A Generalized Künneth Theorem.*—Let (X, B, p) denote a fiber space and Y a topological space. Furthermore, let ξ: $X \times Y \to X$ denote the natural projection. Then $(X \times Y, B, p\xi)$ is a fiber space.

THEOREM. *The spectral sequences* $\{E_j(X, H(Y, G)), d_j\}$ *and* $\{E_j(X \times Y, G), d_j\}$ *are isomorphic, i.e., there exist isomorphisms*

$$\alpha_j: E_j(X, H(Y, G)) \to E_j(X \times Y, G)$$

such that $d_j\alpha_j = \alpha_j d_j$.

In the sequel we will identify the above spectral sequences by reason of this theorem.

2.4. *Cap Products.*—Suppose that G_1, G_2 are coefficient groups paired to G. If X is a space and c: $C_q(X) \to G_1$ is a q-cochain and $u \otimes g_2 \in C_{p+q}(X, G_2)$ is a singular $p + q$ simplex with g_2 as coefficient, we set

$$c \cap (u \otimes g_2) = u^p \otimes c({}^q u)g_2 \in C_p(X, G),$$

where u^p is the p-face of u determined by the last $p + 1$ vertices $v_q, \ldots, v_{p+q}$ of Δ^{p+q} and ${}^q u$ is the q-face of u determined by the first q vertices $v_0, \ldots, v_q$. This

pairing of $C^q(X, G_1)$ and $C_{p+q}(X, G_2)$ to $C_p(X, G)$ induces a pairing of $H^q(X, G_1)$ and $H_{p+q}(X, G_2)$ to $H_p(X, G)$ called the *cap product*. As usual, $h^q \cap h_{p+q}$ denotes the element of $H_p(X, G)$ to which h^q and h_{p+q} are paired. This pairing differs by a factor of $(-1)^{p+q}$ from that given by Eilenberg.[6]

2.5. *Pairing $\pi_r(B)$ and $H(F, G)$ to $H(F, G)$.*—Let (X, B, p) denote a fiber space with fiber $F = p^{-1}(b)$. If λ is a lifting function[4] for (X, B, p), λ induces a map $\tilde{\lambda}$: $\tilde{B} \times F \to X$, where $\tilde{B}$ is the space of paths in B emanating from b. For $\omega \in \tilde{B}$, $f \in F$, set

$$\tilde{\lambda}(\omega, f) = \lambda(f, \omega)(1).$$

Then, if Λ is the loop space of B at b, $\tilde{\lambda}$: $\Lambda \times F \to F$. Hence, employing the Künneth theorem, $\tilde{\lambda}$ induces

$$\lambda_*: H_{r-1}(\Lambda) \otimes H_n(F) \to H_{n+r-1}(F).$$

Thus $H_{r-1}(\Lambda)$ and $H(F)$ are paired to $H(F)$. Now we identify $\pi_r(B)$ with $\pi_{r-1}(\Lambda)$, and thus the pairing of $\pi_r(B)$ and $H_n(F)$ to $H_{n+r-1}(F)$ is obtained by using the Hurewicz homomorphism $\pi_{r-1}(\Lambda) \to H_{r-1}(\Lambda)$.

3. THE BASIC MAPS

3.1. *The Map φ.*—Let B denote a 0-connected space, and fix $b \in B$. Furthermore, let $\tilde{B}$ denote the space of paths in B emanating from b. Then, if ξ: $\tilde{B} \to B$ is given by $\xi(\omega) = \omega(1)$, $(\tilde{B}, B, \xi)$ is a fiber space, with fiber $\Lambda = p^{-1}(b)$ the space of loops. Let $\hat{C}(\tilde{B})$ denote the singular chains of $\tilde{B}$ based on prisms and $C(B)$ the singular chains of B based on simplexes. Then, using the notation of section 2.2, set $\tilde{A} = A(\tilde{B})$, $\tilde{A}^p = A^p(\tilde{B})$. $\tilde{A}$ is then filtered, and we have an associated spectral sequence $\{E_j(\tilde{B}), d_j\}$. Our objective in this section is a dimension-preserving homomorphism (not, in general, a chain map) φ: $C(B) \to \tilde{A}$. Therefore, let s: $\Delta^n \to B$ denote a singular n-simplex. Then define $\tilde{s}$: $\Delta^n \times \Delta^n \to \tilde{B}$ by $\tilde{s}(x, y) = s(\overrightarrow{v_0x})s(\overrightarrow{xy})$, $(x, y) \in \Delta^n \times \Delta^n$, where v_0 is the leading vertex of Δ^n, and $\overrightarrow{v_0x}$, $\overrightarrow{xy}$ are line segments with indicated end points. For $0 \leq q \leq n$, let ${}^q\Delta^n$ and $\Delta^{n,\,n-q}$ denote q and $n - q$ faces of Δ^n determined, respectively, by the first q vertices of Δ^n and the last $n - q$ vertices of Δ^n. Let $\beta_q{}^n$: $\Delta^q \times \Delta^{n-q} \to {}^q\Delta^n \times \Delta^{n,\,n-q}$ denote the natural barycentric map. Then set $\varphi^q(s) = \tilde{s}\beta_q{}^n$, and note that $\varphi^q(s) \in \tilde{A}^{n-q}$. Finally, set $\varphi(s) = \sum \varphi^q(s)$. We note here that φ depends on n, but this fact will not be displayed in the notation. Thus φ: $C(B) \to \tilde{A}$ is a dimension-preserving homomorphism. φ can also be interpreted as arising from a chain approximation to the diagonal map $\Delta^n \to \Delta^n \times \Delta^n$ as follows: Interpreting Δ^n as a finite complex with ordered vertices in the usual manner, let σ^p denote a p-face of Δ^n, and ${}^q\sigma^p$, $\sigma^{p,\,p-q}$ faces of σ^p spanned by the first $q + 1$ and last $p - q + 1$ vertices, respectively. Then the correspondence

$$\sigma^p \to \sum_{q=0}^{p} {}^q\sigma^p \times \sigma^{p,\,p-q}$$

gives rise to a chain mapping D: $C(\Delta^n) \to C(\Delta^n \times \Delta^n)$, which is a chain approximation to the diagonal map in the sense of Lefshetz. Then $\varphi(s)$ can be given by restricting $\tilde{s}$ to the simplicial chain $D(\Delta^n)$ in the product complex $\Delta^n \times \Delta^n$.

3.2. *The Basic Lemma.*—Suppose that s is a singular n-simplex in B whose $r-1$ skeleton lies at $b \in B$, $1 \leq r \leq n$. Let ${}^r s$ denote the r-face of s given by the composition $\Delta^r \to \Delta^n \xrightarrow{s} B$, where the first map is the injection $\Delta^r \subset \Delta^n$. Furthermore, we denote by ${}^r\tilde{s}$ the $r-1$ singular simplex in Λ given by the composition $\Delta^{r-1} \to \Delta^{r, r-1} \to \Lambda$, where the first map is the natural barycentric map and the second map assigns to each $x \in \Delta^{r, r-1}$ the arc $\overrightarrow{{}^r s(v_0 x)}$ from the leading vertex v_0 to x. ${}^r\tilde{s}$ determines an element of $\pi_{r-1}(\Lambda)$ which we denote by $[{}^r\tilde{s}]$. Then the following lemma is valid.

LEMMA. *If s is a singular n-simplex in B whose $r-1$ skeleton ($1 < r \leq n$) lies at $b \in B$, then*

$$\partial\varphi(s) - \varphi\partial(s) = \alpha - \beta \bmod \bar{A}^{n-r-1},$$

where α and β are singular $(r-1, n-r)$ prisms in $\bar{A}^{n-r}$ having the following properties:

(i) $B\alpha = B\beta = s^{n-r}$, the $n-r$ face of s determined by the last $n-r+1$ vertices of Δ^n.

(ii) $\Lambda\alpha \in [{}^r\tilde{s}]$; $\Lambda\beta$ is degenerate, having the constant loop at b as constant value.

Of course, in the above lemma, if $r > n$, $\partial\alpha(s) = \varphi\partial(s)$ trivially. On the basis of the above lemma, the following theorem is valid.

THEOREM. *If B is $r-1$-connected, $r > 1$, the map φ: $C(B) \to \bar{A}$ induces*

$$\varphi_1\colon C(B, G) \to E_1(\bar{B}, G),$$
$$\varphi_i\colon H(B, G) \to E_i(\bar{B}, G), \qquad i = 2, \ldots, r,$$

such that $d_1\varphi_1 = \varphi_1\partial$ and $d_i\varphi_i = 0$, $i = 2, \ldots, r-1$.

3.3 *The Map ψ.*—Let (X, B, p) denote a fiber space with B $r-1$-connected and $F = p^{-1}(b)$ a fixed fiber. If λ is a lifting function for (X, B, p), λ induces a map $\tilde{\lambda}$: $\bar{B} \times F \to X$ (see sec. 2.5). If η: $\bar{B} \times F \to \bar{B}$ is the natural projection, then $(\bar{B} \times F, B, \xi\eta)$ is a fiber space, and $p\tilde{\lambda} = \xi\eta$. Therefore, $\tilde{\lambda}$ induces homomorphisms

$$\lambda_i\colon E_i(\bar{B} \times F, G) \to E_i(X, G)$$

such that $d_i\lambda_i = \lambda_i d_i$, $i \geq 1$. Now, applying the theorem of section 3.2 with $G = H(F, G)$ and identifying $E_i(\bar{B}, H(F, G))$ with $E_i(\bar{B} \times F, G)$, we set $\psi_i = \lambda_i\varphi_i$, $1 \leq i \leq r$. Therefore, ψ_1: $C(B, H(F, G)) \to E_1(X, G)$ and ψ_i: $H(B, H(F, G)) \to E_i(X, G)$ for $2 \leq i \leq r$. It is known that $C(B, H(F, G))$ is isomorphic to $E_1(X, G)$, and it is not difficult to show that ψ_1 is such an isomorphism, where the inverse of ψ_1 is induced by the homomorphism θ of section 2.2. Therefore, this fact, together with the theorem of section 3.2, implies that the ψ_i are isomorphisms for $1 \leq i \leq r$ and $d_i = 0$ for $2 \leq i \leq r-1$.

3.4. LEMMA. *If (X, B, p) and (X', B, p') are fiber spaces over B, which is $r-1$-connected, and if f: $X \to X'$ is a fiber-preserving map, then the following diagram is commutative:*

$$\begin{array}{ccc} H(B, H(F, G)) & \xrightarrow{f_*} & H(B, H(F', G)) \\ \downarrow{\scriptstyle \psi_r} & & \downarrow{\scriptstyle \psi_{r'}} \\ E_r(X, G) & \xrightarrow[f_r]{} & E_r(X', G) \end{array}$$

where F, F' are fibers over $b \in B$, the f's are naturally induced by f, and ψ_r, ψ_r' are the maps defined in section 3.3.

4. THE STRUCTURE OF d_r

Consider again the fiber space $(\tilde{B}, B, \xi)$, B $r-1$-connected. Applying section 3.3, we have isomorphisms $\tilde{\psi}_1$: $C(B, H(\Lambda)) \to E_1(\tilde{B})$ and $\tilde{\psi}_i$: $H(B, H(\Lambda)) \to E_i(\tilde{B})$, $2 \leq i \leq r$. Let φ: $C(B) \to \bar{A}$ denote the map of section 3.1. Then, if s is a singular n-simplex in B, $\partial\varphi(s) - \varphi\partial(s)$ is a cycle mod $\bar{A}^{n-r-1}$ and hence determines an element $[\partial\varphi(s) - \varphi\partial(s)]_1 \in E_1^{n-r,\,r-1}(\tilde{B})$. Then, employing the lemma of section 3.2, it is easy to see that $\tilde{\psi}_1(\gamma({}^r s)s^{n-r}) = [\partial\varphi(s) - \varphi\partial(s)]_1$, where $\gamma \in C^r(B, \pi_r(B))$ is the characteristic cocycle of B, ${}^r s$ and s^{n-r} are as in section 3.2, and $\pi_r(B)$ is identified with $H_{r-1}(\Lambda)$. It follows, then, that if z is an n-cycle in $C(B, G)$, then $\partial\varphi(z)$ determines an element $[\partial\varphi(z)]_1 \in E_1^{n-r,\,r-1}(\tilde{B}, G)$ and $\tilde{\psi}_1(\gamma \cap z) = [\partial\varphi(z)]_1$, where the cap product is derived from the pairing of $\pi_r(B)$ and G to $\pi_r(B) \otimes G$, and hence $\gamma \cap z \in C(B, \pi_r(B)) \otimes G) = C(B, H_{r-1}(\Lambda, G)$. Hence we have

$$\tilde{\psi}_r(\tilde{\gamma} \cap h) = d_r\varphi_r(h),$$

where $\tilde{\psi}_r$: $H(B, H(\Lambda, G)) \to E_r(\tilde{B}, G)$, φ_r: $H(B, G) \to E_r(\tilde{B}, G)$, and $\tilde{\gamma}$ is the characteristic cohomology class of B. Now let $G = H(F)$ in the preceding, and identify $H(B, H(\Lambda, H(F)))$ with $H(B, H(\Lambda \times F))$ and $E_r(\tilde{B}, H(F))$ with $E_r(\tilde{B} \times F)$. Then, if λ_r: $E_r(\tilde{B} \times F) \to E_r(X)$ is the homomorphism of section 3.3 induced by a lifting function λ, we have

$$\lambda_r\tilde{\psi}_r(\tilde{\gamma} \cap h) = \lambda_r d_r\varphi_r(h).$$

Now $\lambda_r d_r\varphi_r = d_r\lambda_r\varphi_r = d_r\psi_r$, where ψ_r: $H(B, H(F)) \to E_r(X)$, and hence $\lambda_r d_r\varphi_r(h) = d_r\psi_r(h)$. On the other hand, applying section 3.4,

$$\lambda_r\tilde{\psi}_r(\tilde{\gamma} \cap h) = \psi_r\lambda_*(\tilde{\gamma} \cap h),$$

where

$$\lambda_*\colon H(B, H(\Lambda \times F)) \to H(B, H(F))$$

is induced by $\tilde{\lambda}$: $\Lambda \times F \to F$. But it is clear that $\lambda_*(\tilde{\gamma} \cap h)$ is then just the cap product $\tilde{\gamma} \cap h$, where $\pi_r(B)$ $(= H_{r-1}(\Lambda))$ and $H(F)$ are paired to $H(F)$ according to section 2.5. Therefore,

$$\psi_r(\tilde{\gamma} \cap h) = d_r\psi_r(h), \qquad h \in H(B, H(F)).$$

Since ψ_r is an isomorphism, we may identify $E_r(X)$ with $H(B, H(F))$, and hence d_r: $E_r(X) \to E_r(X)$ is given by $d_r(h) = \tilde{\gamma} \cap h$. Thus our outline is complete for the proof of the main result with integral coefficients. The use of an arbitrary coefficient group offers no additional difficulties.

* This paper was written by the junior author following the untimely death of Professor Hurewicz. It was supported, in part, by the Wisconsin Alumni Research Foundation.

[1] J. P. Serre, *Ann. Math.*, **54**, 425–505, 1951.

[2] W. Hurewicz and E. Fadell, these PROCEEDINGS, **41**, 961–964, 1955.

[3] The junior author has also proved this theorem for fiber spaces in the sense of Serre.

[4] W. Hurewicz, these PROCEEDINGS, **41**, 956–961, 1955.

[5] An alternate proof of the theorem based on cubical singular chains will appear in the forthcoming "Symposium in Algebraic Topology" publication (Mexico City, 1956).

[6] S. Eilenberg, *Ann. Math.*, **45**, 407–447, 1944.

INTEGRATION OF A DIFFERENTIAL FORM ON AN ANALYTIC COMPLEX SUBVARIETY

By Pierre LeLong

UNIVERSITY OF PARIS*

Communicated by Marston Morse, December 12, 1956

I. The purpose of this note is to give a precise definition of the operator of integration

$$t(\varphi) = \int_W \varphi \tag{1}$$

for an exterior differential form φ on an analytic complex subvariety W. The problem arises because an analytic complex subvariety in a domain D of C^n (or, more briefly, an *analytic set* in D) is not, in general, a manifold. We give (a) an existence theorem for $t(\varphi)$ and (b) a proof that t is a closed current in D, that is, $t(\psi) = 0$ for the forms ψ with compact support, which are homologous to zero in D.

II. A set W is called an *analytic set* in a domain D of $C^n(z_1, \ldots, z_n)$ if $M \in D$ has a neighborhood (U_M) such that $(U_M) \cap W$ is defined by the simultaneous equations

$$f_1(z_1, \ldots, z_n) = 0, \ldots, f_\nu(z_1, \ldots, z_n) = 0, \tag{2}$$

where f_k is holomorphic in (U_M); $M \in W$ is an *ordinary point* of W if there exists an analytic one-to-one mapping $Z = F(z)$ such that $F[(U_M) \cap W] = F[(U_M)] \cap C^s$, where C^s is a complex linear subspace. We denote by A^q a complex q-vector given by

$$z_k = z_k' + \sum_i a_k^i u_i, \qquad 1 \leq k \leq n,\ 1 \leq i \leq q, \tag{3}$$

with unitary representation and fundamental form

$$d\tau_{2q} = \Omega(A^q) = \left(\frac{i}{2}\right)^q (-1)^{q(q-1)/2}\, du_1 \wedge d\bar{u}_1 \ldots du_q \wedge d\bar{u}_q.$$

If s is the maximum of the numbers q such that P can be an isolated point of $W \cap A^q$, $p = n - s$ is the complex dimension of W in $P \in W$. We have the decomposition $W = \bigcup W^k$, where W^k is k complex dimensional in each of its points. If $W' = W \cap A^q$ is not of dimension zero, the boundary of $B(P, r) = E\left[\sum_1^q |u_i|^2 < r^2\right]$ intersects[1] W'. Starting from this property and applying the Kronecker integral to the system (2) after substituting equation (3), where z_k' and a_k^i are considered as complex parameters, we obtain

Theorem 1. *If W is an analytic set in D and $M(z_k^0)$ is an isolated point of $A_0^q \cap W$, where A_0^q is given by $(a_i^k)_0$ and $z_k' = z_k^0$ in equation (3), there exists a neighborhood (U_M) of M, $\epsilon > 0$ and $\epsilon' > 0$, such that for $|a_i^k - (a_i^k)_0| < \epsilon$, and $|z_k' - z_k^0| < \epsilon'$, $U_M \cap W \cap A^q$ is a set of l ($\leq N(M_0)$) isolated points, where $N(M_0)$ is the number of intersections of $A_0^q \cap W$ in M_0.*

III. *Positive Currents.*—We denote by $^*\psi$ the adjoint of a form ψ; $^*\Omega(A^q)$ is the fundamental form of the A^{n-q} orthogonal to A^q.

Definition: A *current*[2] t is called *positive* of degree q (or belongs to Θ_q^+) if t is homogeneous of degree (q, q) in dz_i, $d\bar{z}_j$ and if for every complex q-vector A^q, the distribution $T[A^q](f) = \int t \wedge {}^*\Omega(A^q)f$ is a positive measure. A *form* φ is called *positive* of degree q (or belongs to Φ_q^+) if φ has continuous coefficients and $\varphi \in \Theta_q^+$.

THEOREM 2. *A positive current* $t = (i/2)^q(-1)^{q(q-1)/2} \sum_{(i)(j)} t_{(i)(j)} dz_{i_1} \wedge \ldots \wedge dz_{i_q} d\bar{z}_{j_1} \wedge \ldots \wedge d\bar{z}_{j_q}$ *is continuous of order zero; the distributions* $T_{(i)(j)}(f) = \int t_{(i)(j)} f \, d\tau_{2n}$ *are complex measures:* $T_{(i)(j)} = \bar{T}_{(j)(i)}$ *and* $T_{(k)(k)} \geq 0$.

Remark A: We put $|t|_D = \sup |t(\varphi)|$ for φ, (C^∞), with compact support $K(\varphi) \subset D$, and $|\varphi_{(i)(j)}| \leq 1$ for the coefficients of φ. Then it is possible to find a system Λ of $(C_n^q)^2$ complex q-vectors A^q, with $|a_i^k - (a_i^k)_0| < \epsilon$, where $\epsilon > 0$ and $A_0^q[(a_i^k)_0]$ are given, such that we have $|t|_D \leq k(\Lambda) \sup_s T[A_s^p]$, where $k(\Lambda)$ is a constant depending on Λ. The class of the positive currents possesses the following properties:

THEOREM 3 (multiplication). *If* $t \in \Theta_p^+$ *and* $\varphi \in \Phi_1^+$, *then* $t \wedge \varphi \in \Theta_{p+1}^+$.

THEOREM 4 (division). *If* $t \wedge \varphi = 0$, $t \in \Theta_p^+$, *and if* $\varphi \in \Phi_1^+$ *satisfies* $\varphi^q \not\equiv 0$ *and* $\varphi^{q+1} = 0$ *in* D, *then we have* $t = t_1 \wedge \varphi^q$, *and* $t_1 \in \Theta_{p-q}$ $(t_1 = 0$ *if* $p < q)$.

THEOREM 5. *The image of a positive current induced by an analytic and locally one-to-one transformation is a current which is positive.*

IV. If W is the analytic set $f(z_1, \ldots, z_n) = 0$ in D, we have the following two expressions for the current $t(\varphi)$:

$$(a) \quad t = \frac{2}{\pi}\left[\frac{i}{2} d_z d_{\bar{z}} \log |f|\right]; \qquad (b) \quad t = \theta_1 \frac{i}{2} df \wedge d\bar{f}.$$

In (a) the bracket denotes the positive current relative to the plurisubharmonic function $\log |f|$. On the other hand, (b) is available only for forms φ whose support $K(\varphi)$ contains only ordinary points of W; in (b) the positive current θ_1 of degree zero (measure density) is given by an analytic locally one-to-one mapping $Z = F(z)$ of W on a complex subspace.

We consider now an analytic set W^p of complex dimension p irreducible in D and denote by W_0^p the connected manifold of the ordinary points of W^p, and we put $E_1 = W^p - W_0^p$. The definition of the current $t(\varphi)$ (the integral of φ on W^p) will be given in three steps: (1) Definition of the positive current $t_0(\varphi) = \int_{W_0^p} \varphi$ on φ with support $K(\varphi) \subset D - E_1$. (2) Majorization of the norm of t_0 in a neighborhood of $M \in W^p$, for instance in a sphere $B(M, r)$. (3) Solving of a continuation problem for t_0 defined in $D - E_1$, to obtain t defined in D.

1. If $M \in W_0^p$, t_0 is obviously defined by an analytic mapping of $W^p \cap (U_M)$ in a complex subspace.

2. We use the following result, which is a consequence of Theorem 1 and Remark A:

THEOREM 6. *If* W *is an analytic set in* D, *of complex dimension* p *in every* $M \in W$, *and* $D_1 \subset\subset D$ *is a compact domain in* D, *there exists a constant* $\lambda(D_1) > 0$ *such that the norm* $|t_0|_M^r$ *of the current* t_0 *in the spheres* $B(M, r) \subset D_1$ *satisfies* $|t_0|_M^r < \lambda(D_1) r^{2p}$.

V. *Continuation of a Closed Current.*—Given a current t in a domain $D - E$, where $D \subset R^m(x_1, \ldots, x_m)$ and E is the subspace $x_1 = \ldots = x_s = 0$, a necessary condition that t be continuable to the forms φ with support $K(\varphi) \subset D$ is the

convergence of $\sum_i t \wedge \alpha_i\varphi$, $K(\alpha_i) \subset D - E$, where α_i is a partition of unity in $D - E$. We give now conditions that a closed current t have a continuation $\bar{t}$ by a closed current. We denote by $\alpha_r(x_1, \ldots, x_s)$ a kernel (C^∞), with $0 \leq \alpha_r \leq 1$; $\alpha_r = 1$ in a neighborhood ω_r of the origin ω_0 $(x_1 = \ldots = x_s = 0)$; α_r has a compact support K_r; $\omega_r \subset K_r \subset \omega_r'$, and ω_r' tends to ω_0 when $r \to 0$. We put $\theta = \bar{t} - t$, and suppose that t is homogeneous of degree k.

THEOREM 7. *A necessary and sufficient condition for the existence of $\bar{t}$ is* (a) $\lim_{r=0} \alpha_r t = 0$; (b) *the existence of the limit* $\tau(\varphi) = (-1)^k t[d\alpha_r \wedge \varphi]$, *for a sequence of kernels α_r with properties listed above.*

Thus τ is a current with support in E and is homologous to zero; the limit is independent of the sequence α_r; we have $d\theta = \tau$, $\bar{t} = t + \theta$. If $\tau = 0$, we choose $\theta = 0$, and we say that $\bar{t}$ is the simple extension of t. If $\bar{t}$ exists, t is continuous of finite order q in $K \cap (D - E)$, where K is a compact domain in D. We put $|t|_K^r = \sup |t(\varphi)|$, where φ satisfies $K(\varphi) \subset K \cap \left[\sum_1^s x_i^2 < r^2\right]$ and $|D^{(\alpha)}\varphi_{(i)}| \leq 1$, for each derivative of total order $\leq q$ of the coefficients of φ. We choose $\alpha_r = \alpha(x_i/r)$ and denote $(\partial\alpha/\partial x_j)(x_i/r)$ by $\beta_{j,r}(x_i)$, to obtain more precise sufficient conditions:

THEOREM 8. *A sufficient condition for the existence of $\bar{t}$ is the existence of a finite number L such that we have* $|t\beta_{j,r}(x_i)|_K^r \leq Lr$.

COROLLARY. *If t is continuous of order zero and if* $r^{-1}|t|_K^r \to 0$, *$\bar{t}$ is obtained by the simple extension of t in $K \subset D$.*

VI. Now we consider $E_1 = W^p - W_0{}^p$ and put $E_1 = W_1 \cup W_2 \cup \ldots \cup W_s$, where W_k is the manifold of the ordinary points of $E_1 - [W_1 \cup W_2 \cup \ldots \cup W_{k-1}]$; W_k is a manifold of complex dimension $\nu_k \leq p - k$. In $M \in W_k$, we consider a neighborhood (U_M) and an analytic one-to-one mapping $Z = F(z)$ such that $F[(U_M) \cap W_k] = F[(U_M) \cap C^{\nu k}]$ where $C^{\nu k}$ is a complex subspace. By Theorems 5 and 6 and the corollary to Theorem 8, we obtain the main result of this note:

THEOREM 9. *If W^p is an analytic set in a domain D of C^n and if W^p is of complex dimension p in each of its points, the positive current of degree (p, p) defined by*

$$t_0 = \int_{W_0{}^p} \varphi$$

(where $W_0{}^p$ is the manifold of the ordinary points of W^p, and $E_1 = W^p - W_0{}^p$) is convergent in $D - E_1$. The simple extension t of t_0 gives a closed positive current $t(\varphi)$ on the forms φ with compact support in D.

If W is not homogeneous p complex dimensional in D, the current $t(\varphi) = \int_W \varphi$ is a finite sum of homogeneous, positive closed currents t_k in D whose supports are the components of W in the decomposition $W = \cup W^k$, where W^k is k complex dimensional in each of its points.

* Written while the author was at the Institute for Advanced Study (Princeton, N.J.) under the sponsorship of the Air Force Office of Scientific Research.

[1] Cf. R. Remmert and K. Stein, *Math. Ann.*, **126**, 263–336, 1953, in particular p. 283. The notion of complex dimension is invariant under one-to-one complex analytic mappings.

[2] Cf. G. de Rham, *Variétés différentiables* (Paris: Herrmann & Cie, 1955). A current t is continuous of order s if $t(\varphi_n) \to 0$ for every sequence of forms φ_n, (C^∞), with compact supports $K(\varphi_n) \subset K_0$, and $\sup |D^{(\alpha)}\varphi_{n,(i)}| \to 0$, for every coefficient $\varphi_{n,(i)}$ and every derivative (α) of total order $\leq s$.

APPLICATION OF HILBERT SPACE METHODS TO LIE GROUPS ACTING ON A DIFFERENTIABLE MANIFOLD

By Jacqueline LeLong-Ferrand*

UNIVERSITY OF PARIS

Communicated by Deane Montgomery, December 17, 1956

1. M^n will denote an orientable connected manifold of dimension n, not necessarily finite, but admitting a completion $\bar{M}^n$ by a boundary ∂M^n, and satisfying the following conditions:

(*a*) The functions defining the change of local co-ordinates possess Lipschitz continuous derivatives of the first order.

(*b*) Every $x \in \partial M^n$ admits a neighborhood V homeomorphic to the half-cell: $[|x^i| < 1 \text{ for } i = 1, 2, \ldots, n-1;\ 0 \leq x^n < 1]$, of R^n, this homeomorphism defining an admissible co-ordinate system such that the points of $\partial M^n \cap V$ are characterized by $x^n = 0$.

Every Lipschitz continuous vector field ξ on M^n defines an infinitesimal transformation X_ξ (or X when no confusion is possible) acting on the space $\mathfrak{L}_{pq}$ of Lipschitz continuous tensors T of type (p, q) according to the law

$$X_\xi T^{j_1 \ldots j_q}_{i_1 \ldots i_p} = \xi^r \frac{\partial}{\partial x^r} T^{j_1 \ldots j_q}_{i_1 \ldots i_p} + \sum_{k=1}^{q} \frac{\partial \xi^r}{\partial x^{i_k}} T^{j_1 \ldots j_q}_{i_1 \ldots r \ldots i_p} - \sum_{h=1}^{q} \frac{\partial \xi^{j_h}}{\partial x^r} T^{j_1 \ldots r \ldots j_q}_{i_1 \ldots i_p}$$

where the ξ^i denote the local components of ξ with respect to the co-ordinate system x^i; the components of $X_\xi T$ are bounded, measurable, and almost everywhere defined. In particular, X acts on the space $\mathfrak{L}$ of Lipschitz continuous functions f according to the law $Xf = \xi^i \partial f / \partial x^i$, and on the space $\mathfrak{L}_p$ of Lipschitz continuous differential forms of degree p, $\varphi = \varphi_{i \ldots i_p} dx^{i_1} \wedge \ldots \wedge dx^{i_p}$, considered as defined by p-covariant antisymmetric tensors $\varphi_{i_1 \ldots i_p}$.

More generally ν vector fields ξ_α $(\alpha = 1, 2, \ldots, \nu)$ define a ν-dimensional infinitesimal group G of transformations of M^n if the corresponding operators X_α satisfy the structural equations

$$[X_\alpha X_\beta] = X_\alpha X_\beta - X_\beta X_\alpha = C_\alpha{}^\gamma{}_\beta X \qquad (C_\alpha{}^\gamma{}_\beta = Ct).$$

Then any linear combination $X = \sum e_\alpha X_\alpha$ with constant coefficients e_α defines an operator of G.

2. If a Riemannian metric $ds^2 = g_{ij}\, dx^i dx^j$ can be defined on $\bar{M}^n$, the g_{ij} being Lipschitz continuous, we set

$$(S, T) = g^{i_1 r_1} g^{i_2 r_2} \ldots g^{i_p r_p} g_{j_1 s_1} g_{j_2 s_2} \ldots g_{j_q s_q} S^{j_1 \ldots j_q}_{i_1 \ldots i_p} \bar{T}^{s_1 \ldots s_q}_{r_1 \ldots r_p},$$

where S, T are two tensors of the same type (p, q) and $\bar{T}$ denotes the complex conjugate of T; we define a global scalar product by setting

$$\langle S, T \rangle = \int_{M^n} (S, T)\, d\tau, \qquad \text{where } d\tau = \sqrt{g}\ dx^1 \wedge \ldots \wedge dx^n,$$

and denote by $\mathfrak{IC}_{pq}$ the Hilbert space of square-summable tensors T of type (p, q) (satisfying $\|T\|^2 = \langle T, T \rangle < \infty$); then, to every infinitesimal transformation X, related to a vector field ξ, we make correspond a new operator $\tilde{X}$ by the formula

$$XT + \bar{X}T = \sum_{k=1}^{p} t^{r}{}_{i_k} T^{j_1 \ldots j_q}_{i_1 \ldots r \ldots i_q} - \sum_{h=1}^{p} t_r{}^{j_h} T^{j_1 \ldots r \ldots j_h}_{i_1 \ldots i_p} + t^{r}{}_{r} T^{j_1 \ldots j_q}_{i_1 \ldots i_p},$$

where $t_{ij} = Xg_{ij} = \xi_{i,j} + \xi_{j,i}$ and $t^{r}_{s} = g^{rs}t_{is}$.

If only an element of volume $d\tau = \sqrt{g}\, dx^1 \wedge \ldots \wedge dx^n$ is given on M^n, we restrict our considerations to the Hilbert space $\mathfrak{H} = \mathfrak{H}_{00}$ of square-summable functions f and set

$$\wedge f, g \wedge = \int_{M^n} f\bar{g}\, d\tau; \quad \|f\|^2 = \wedge f, f \wedge;$$

$$Xf + \bar{X}f = f\delta\xi, \qquad \text{with } \delta\xi = -\frac{1}{\sqrt{g}} \frac{\partial}{\partial x^i} (\sqrt{g}\xi^i).$$

Then we have the following fundamental results:

(2*a*) $<XS, T> = <S, \bar{X}T>$ *for any two tensors* $S, T \in \mathfrak{L}_{pq}$ [resp. for any two functions $S, T \in \mathfrak{L}$], *of which one at least has a compact support in* M^n, *if, and only if the vector field* ξ *is tangent to* ∂M^n; we shall express this last condition by saying that ∂M^n is *globally invariant.*

(2*b*) $Xf + \bar{X}f = 0$ *for every* $f \in \mathfrak{L}$ *if, and only if,* X *preserves the volumes,* this last condition being possibly written $\delta\xi = 0$ or $Xd\tau = 0$.

(2*c*) $XT + \bar{X}T = 0$ *for every* $T \in \mathfrak{L}_{pq}$ *if, and only if,* X *is an infinitesimal isometry,* or, in other terms, *if* ξ *is a Killing vector field.*

(2*d*) *If* X *is an infinitesimal conformal transformation, we have*

$$XT + \bar{X}T = \left[1 + \frac{2}{n}(q - p)\right] \delta\xi\, T.$$

3. In all further developments, we shall denote by X [resp. $\bar{X}$] the closure of the former operator X [resp. $\bar{X}$], considered as acting on $\mathfrak{H}_{pq}$; the domains of these operators are dense in $\mathfrak{H}_{pq}$, and we easily see that each operator X, $\bar{X}$, is contained in the adjoint of the other.

G being a ν-dimensional infinitesimal group on M^n, we say that ∂M^n is globally invariant under G, if ∂M^n is globally invariant under any $X \in G$, and we denote by $\mathfrak{H}_{pq}{}^0$ the closed subspace of $\mathfrak{H}_{pq}$ constituted by invariant tensors T of type (p, q) (satisfying $XT = 0$ for any $X \in G$). Then, if $\mathfrak{H}_{pq}{}^1$ is the orthogonal complement of $\mathfrak{H}_{pq}{}^0$ and if $\{X_\alpha\}$ denotes a basis of G, it can be easily seen that the direct sum $\oplus \bar{X}_\alpha \mathfrak{H}_{pq}$ is dense in $\mathfrak{H}_{pq}{}^1$. This yields the "weak" decomposition formula

$$T = T_0 + T_1, \tag{3a}$$

where $T_0 \in \mathfrak{H}_{pq}{}^0$ and T_1 is the strong limit, for $k = \infty$, of a sequence $T^k = \sum_\alpha \bar{X}_\alpha T_\alpha{}^k$. And in the particular case of a global 1-parameter compact group of isometries, the results of section 4 will carry the "strong" decomposition formula

$$T = T_0 + XS_1 \qquad [T_0 \in \mathfrak{H}^0_{pq},\ S_1 \in \mathfrak{H}_{pq0}]. \tag{3b}$$

These formulas present a special interest for differential forms because of their analogy with Kodaira's decomposition formula and because of the relations of permutability $dX = Xd$, $\delta\bar{X} = \bar{X}\delta$. Among the applications of relation (3*a*), we have

THEOREM 1. *On a finite manifold M^n admitting an infinitesimal group of isometries such that ∂M^n is globally invariant, every invariant form, homologous* [resp. cohomologous] *to zero, is the exact differential* [resp. codifferential] *of an invariant form.*

4. If $\mathcal{Y}$ is a compact connected 1-parameter group of isometries of M^n, such that ∂M^n is globally invariant, and if $x \to tx$ denotes the transformation of M^n corresponding to any $t \in \mathcal{Y}$, we may consider the operator Y_4: $T(x) \to T(tx)$ as defining a unitary representation of $\mathcal{Y}$ in $\mathfrak{H}_{pq}$, and, according to Stone's theorem, we have the spectral decomposition

$$Y_4 = \sum_{-\infty}^{+\infty} e^{in\omega t} E_n, \tag{4a}$$

where E_n is a complete set of projections on mutually orthogonal spaces, E_0 agreeing with the projection on $\mathfrak{H}_{pq}{}^0$. Formula (4*a*) implies

$$X = \sum_{-\infty}^{+\infty} in\omega E_n, \tag{4b}$$

where X is the infinitesimal transformation of $\mathcal{Y}$, and formula (4*b*) proves that every $T \in \mathfrak{H}_{pq}{}^1$ satisfies

$$\|T\| \leq k\|XT\| \qquad \text{with } k = |\omega|^{-1}. \tag{4c}$$

Inequality (4*a*) is a particular case of a more general result, which we prove by another argument based on Koszul's theory[3] of $\mathcal{Y}$-regular manifolds, and may be stated as follows:

THEOREM 2. *If $\mathcal{Y}$ is a compact ν-dimensional Lie group, and, if X_α ($\alpha = 1, 2, \ldots, \nu$) denotes a basis of its algebra, there exists a constant k such that $f \in \mathfrak{H}$ and $\int_G f\, d\tau = 0$ imply $\|f\|^2 \leq k\Sigma\|X_\alpha f\|^2$, where $d\tau$ is the invariant measure.*

THEOREM 3. *If $\mathcal{Y}$ is a compact ν-dimensional Lie group of isometries acting on differentiable manifold M^n such that ∂M^n is globally invariant, and if X_α denotes a basis of its algebra, there exists a constant k, depending only on the integers p, q, such that*

$$X \in \mathfrak{H}_{pq}{}^1 \qquad \text{implies} \qquad \|T\|^2 \leq k\Sigma\|X_\alpha T\|^2.$$

Now, in the case of 1-parameter groups, we prove that the property involved by reaction (4*c*) is characteristic of compact groups. More precisely, we have

THEOREM 4. *An element X of the Lie algebra of a Lie group $\mathcal{Y}$ defines a 1-parameter compact subgroup of $\mathcal{Y}$ if, and only if, there exists a constant k such that every function $f \in \mathfrak{H}$, orthogonal to the space $\mathfrak{H}^1 = \{\varphi;\ \varphi \in \mathfrak{H},\ X\varphi = 0\}$, satisfies*

$$\|f\| \leq k\|Xf\|.$$

THEOREM 5. *In order that a Killing vector field ξ, on a Riemannian manifold M^n, such that ξ is tangent to ∂M^n, define a compact 1-parameter group, it is necessary and sufficient that there exist a constant k such that every function $f \in \mathfrak{H}$, orthogonal to the space $\{\varphi;\ \varphi \in \mathfrak{H},\ X\varphi = 0\}$, satisfies*

$$\|f\| \leq k\|X_\vartheta f\|.$$

The complete proofs will appear subsequently.

* Written while the author was at the Institute for Advanced Study (Princeton, N.J.), under the sponsorship of the Air Force Office of Scientific Research.

References not mentioned in text: K. O. Friedrichs, "The Identity of Weak and Strong Extensions of Differential Operators," *Trans. Am. Math. Soc.*, **55**, 132–151, 1944; S. Kobayashi, "Espaces à connexion de Cartan complets," *Proc. Japan Acad.*, **30**, 709–710, 1954.

[1] J. L. Koszul, "Sur certains groupes de transformations de Lie," in *Colloque de géométrie différentielle* (Strasbourg, 1953), pp. 137–141.

ERRATA: DIOPHANTINE EQUATIONS IN CERTAIN RINGS

In the article of the above title appearing in these PROCEEDINGS, **42**, 656–665, 1956, the following corrections should be made:

Page 656

In the first line below relation (4) read "finite" in lieu of "definite."

In the first line below relation (4*a*) read "$\sigma_{s+1} \equiv 0 \pmod{\mathfrak{p}}$" in lieu of "$\sigma_{s+1} = 0$,"

In the same line read "$\sigma_{s+1} \not\equiv 0 \pmod{\mathfrak{p}}$" in lieu of "$\sigma_{s+1} \neq 0$."

Page 657

In relation (5*a*) read "$s + 1$" in lied of "$s = 1$" above the summation sign.

In the second line below relation (5*a*) read "ρ" instead of "γ."

Page 658

The first displayed formula should be numbered "(8)."

In the seventh line below relation (11) read "turns" in lieu of "turn."

In the fifteenth line below relation (11) read "has" in lieu of "have."

Page 659

In relation (12) read "$\equiv$" in lieu of "$=$."

In the fourth line below relation (14) insert the word "by" between "mentioned" and "$[i, j]_{cl}$."

Page 660

In relation (19) just below the summation sign on the left read "$i = 0$" in lieu of "$1 = 0$."

In relation (19*a*) read "$j = 0$" in lieu of "$i = 0$" below the summation sign.

Emma Lehmer, who kindly read the manuscript of this article critically after the corrected proof sheets had been sent to the printer, found difficulty in following the writer's statements

$$\sum_{b=1}^{c-1} (d, h_1 - b)_c = l - (d, h_1')_c$$
$$= l - 1,$$

occurring two lines below relation (21). These should be corrected as follows:

In the first equation replace "h_1" by "h'," and "h_1'" by "h'."

In the second equation replace "$= l - 1$" by "> 0."

To prove the last statement, it is sufficient to show that $(d, h') < l$. From relation (17) we have $(d, h') \leq l$. If we have $(d, h') = l$, it then follows that

$$\theta^{d+ic} + \theta^{h'+aic} \equiv 1 \pmod{\mathfrak{p}},$$

with $d \not\equiv 0 \pmod{c}$, for $i = 0, 1, \ldots, l-1$, with the a's in the same range. No two of the a's are congruent modulo l, for that would give

$$\theta^{d+(i-j)c} \equiv 1 \pmod{\mathfrak{p}},$$

which contradicts $d \not\equiv 0 \pmod{c}$. Hence the a's are the integers $0, 1, \ldots, l-1$ in some order. Adding together all the resulting congruences, we have $l \equiv 0 \pmod{\mathfrak{p}}$, contradicting $p^n = 1 + cl$.

Page 663

In the first line below relation (33) insert the word "holds" before the word "with."

In the second line below relation (33) delete the word "holds."

In the second line above relation (35) write "$xy \not\equiv 0 \pmod{p}$" in lieu of "$xy \not\equiv \pmod{l}$."

Page 664

In the first line write "$b \equiv 0 \pmod{\mathfrak{p}}$" in lieu of "$b \not\equiv 0 \pmod{\mathfrak{p}}$."

In the fourth line add the words: "This also holds with the left-hand member a unit."

In the sixth line, in the exponent in the left-hand member, write "m_i'" in lieu of "m_1'."

In the sixth line, in the right-hand member, write "$\mathfrak{a}_i^1$" for "$\mathfrak{a}_i$."

In the first line below relation (37) write "$\equiv 0 \pmod{l}$" in lieu of "zero." On same line write "$\not\equiv 0 \pmod{l}$" in lieu of "$\neq 0$."

In the second line below relation (38) write "$\equiv 0 \pmod{l}$" in lieu of "zero."

Add after the last statement of the theorem the sentence, "Also k_1, k_2, and k_3 are integers."

In the first line of note 6, write "$\not\equiv$" in lieu of "$\neq$."

INFORMATION TO CONTRIBUTORS

THE PROCEEDINGS is the official organ of the NATIONAL ACADEMY OF SCIENCES and of the NATIONAL RESEARCH COUNCIL for the publication of brief accounts of important current researches of members of the ACADEMY and of the COUNCIL and of other American investigators. The PROCEEDINGS will aim especially to secure prompt publication of original announcements of discoveries and wide circulation of the results of American research among investigators in other countries and in all branches of science.

ARTICLES should be brief. The viewpoint should be comprehensive in giving the relation of the paper to previous publications of the author or of others and in exhibiting, where practicable, the significance of the work for other branches of science. Elaborate technical details of the work and long tables of data should be avoided, but authors should be precise in making clear the new results and should give some record of the methods and data upon which they are based.

MANUSCRIPTS should be prepared with a current number of the PROCEEDINGS as a model in matters of form, and should be typewritten in duplicate with double spacing, the author retaining one copy. Illustrations should be confined to text figures of simple character, though more elaborate illustration may be allowed in special instances to authors willing to pay for their preparation and insertion. Particular attention should be given to arranging tabular matter in a simple and concise manner.

MANUSCRIPTS will be accepted only from members of the Academy, who will assume responsibility for the propriety and scientific standards of the paper and for any printing costs in excess of those allowed them by the Academy. Manuscripts should be addressed to *Proceedings of the* NATIONAL ACADEMY OF SCIENCES, Attention Miss Mary D. Alexander, University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois.

GALLEY PROOF will be sent, with the understanding that charges for author's corrections shall be billed to him. Author should therefore make final revisions on the typewritten manuscripts. Page proofs will not be sent.

REPRINTS should be ordered when proof is returned, on the reprint order form which will be inclosed with the proof. The order blank gives a tabular estimation of the cost of reprints; a copy will be supplied at any time upon request.

CONTENTS

Page

BIOCHEMISTRY.—THE METABOLISM OF L-THREONINE AND GLYCINE BY *Escherichia Coli* . . . . . *By Dorothy A. Miller and Sofia Simmonds* 195

BOTANY.—STERILE INDUCTION OF CROWN-GALL TUMORS ON CARROT TISSUES IN VITRO . . . . . *By Richard M. Klein and John L. Knupp, Jr.* 199

CHEMISTRY.—THE STRUCTURE OF ALPHA KERATIN . . . . . *By Maurice L. Huggins* 204

CHEMISTRY.—THE STRUCTURE OF COLLAGEN . . . . . *By Maurice L. Huggins* 209

CHEMISTRY.—THE CONTRIBUTION OF THE α-HELICAL CONFIGURATION TO THE OPTICAL ROTATION OF POLYPEPTIDES AND PROTEINS . . *By Paul Doty and R. D. Lundberg* 213

GENETICS.—CONTRIBUTIONS TO THE THEORY OF THE CONTINUOUS BACTERIAL GROWTH APPARATUS. I. KINETICS OF GROWTH OF HOMOGENEOUS POPULATIONS *By H. Moser* 222

GENETICS.—HETEROSIS AND ELIMINATION OF WEAK HOMOZYGOTES IN NATURAL POPULATIONS OF THREE RELATED SPECIES OF *Drosophila* . . . . . *By Crodowaldo Pavan, Theodosius Dobzhansky, and Antonio Brito da Cunha* 226

MATHEMATICS.—REGULARITY THEOREMS FOR SOLUTIONS OF PARTIAL DIFFERENTIAL EQUATIONS WITH VARIABLE COEFFICIENTS . . . . . *By Felix E. Browder* 234

MATHEMATICS.—TWO RECURSIVELY ENUMERABLE SETS OF INCOMPARABLE DEGREES OF UNSOLVABILITY (SOLUTION OF POST'S PROBLEM, 1944) . . *By Richard M. Friedberg* 236

MATHEMATICS.—A THEOREM ON STABILITY OF COMPLEX STRUCTURES . . . . . *By Alfred Frölicher and Albert Nijenhuis* 239

MATHEMATICS.—ON THE SPECTRAL SEQUENCE OF A FIBER SPACE. II. . . . . *By Witold Hurewicz and Edward Fadell* 241

MATHEMATICS.—INTEGRATION OF A DIFFERENTIAL FORM ON AN ANALYTIC COMPLEX SUBVARIETY . . . . . *By Pierre LeLong* 246

MATHEMATICS.—APPLICATION OF HILBERT SPACE METHODS TO LIE GROUPS ACTING ON A DIFFERENTIABLE MANIFOLD . . . . . *By Jacqueline LeLong-Ferrand* 249

ERRATA.—DIOPHANTINE EQUATIONS IN CERTAIN RINGS . . . . . *By H. S. Vandiver* 252